ESTABLISHING GENETIC AND PHYSIOLOGICAL BASELINES FOR THE BLACK-TAILED PRAIRIE DOG (*Cynomys ludovicianus*)

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The black-tailed prairie dog (*Cynomys ludovicianus*) has experienced dramatic declines over much of its historical range due to habitat loss, plague, poisonings, and shootings. Many populations now occur as isolated genetic relicts. A multiple locus genetic profile was obtained using microsatellite analyses of six polymorphic nucleotide repeats from 319 black-tailed prairie dogs collected from 16 colonies throughout the state of Texas. This assessment revealed that existing populations have sufficient variation at all six loci to verify the usefulness of this approach as a primary genetic tool in conservation and preservation.

The data reveals regional-dependent frequency patterns as well as support for founder/bottleneck effects for several of the 16 sites. Although the prairie dog population in Texas as a whole may appear genetically diverse, considerable genetic divergence has already occurred among the subpopulations (F_{ST} = 0.164). Isolation by distance is supported by genic differentiation analysis ($P < 0.001$) and pairwise correlation analysis between genetic distance and geographic distance ($P < 0.001$).

Prairie dogs from six (COC, LUBA, LUBC, LUBD, LUBE, and TAR) of the original 16 sites have been relocated or exterminated, or were in the process of being relocated. Results indicated the following colonies (COT, DAL, HOW, and HUD) are of sufficient size and possess ample genetic diversity to be characterized as candidate foundation populations for future preservation efforts. The proximity of small colonies (< 20 hectares) such as HEMB, LUBB, and PEC, to other colonies should be examined to

determine if they are isolated or part of a metapopulation. Colonies (HAR, HEMA, and SCH) with low genetic diversity would be ideal candidates for supplementation. Alternatively, these colonies could be relocated or blended with other similar but genetically distinct colonies.

Baselines for healthy, pet prairie dog hematology and blood chemistries were also established. Results signify that data gathered from pet prairie dog blood analyses should be referenced against hematology and blood chemistry baselines established using pet prairie dog subjects.

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CHAPTER 1

INTRODUCTION

Objectives

This study was conducted as part of the Texas Black-Tailed Prairie Dog Conservation and Management Plan (TPW, 2004). The principal mission of the TPW plan is to "develop and initiate a statewide plan that will conserve the black-tailed prairie dog, while simultaneously protecting personal and property rights" (TPW, 2004).

The primary objective of this project was to obtain genetic profiles of black-tailed prairie dogs (*Cynomys ludovicianus*) collected from colonies throughout the state of Texas and to use this information to establish the genetic diversity baseline necessary for continued monitoring of their genetics, and speculating about their health of these populations. Additional benefits can be derived from these data, if they are combined with data from the TPW mapping and size analysis of prairie dog colonies throughout the state. Questions that may be addressed include whether increased colony size, proximity to other prairie dog colonies, and/or increased numbers of adjacent prairie dog colonies from which prairie dogs can emigrate can substantially increase genetic diversity in extant populations

Background

The black-tailed prairie dog (*Cynomys ludovicianus*; hereafter references to "prairie dog" in this dissertation will refer to this species alone unless otherwise specified) is one of five species of prairie dogs found in North America. These other four species include the white-tailed prairie dog (*Cynomys leucurus*), the Utah prairie dog

(*Cynomys parvidens*; found only in Utah), the Gunnison prairie dog (*Cynomys gunnisoni*) and the Mexican prairie dog (*Cynomys mexicanus*; found only in Mexico). Black-tailed prairie dogs inhabit mixed-grass and short-grass prairies of the Great Plains region, currently ranging from southern Saskatchewan, Canada, to northern Mexico, and from eastern Nebraska to the foothills of the Rocky Mountains (Miller and Cully, 2001). These reddish-brown, ground-dwelling squirrels average 30 cm in length and 700 g in weight (Hoogland, 1996). They are diurnal, burrowing rodents whose key characteristic is high sociality organized into family groups termed coteries.

In 1902, C. H. Merriam, director of the U.S. Biological Survey (now the U.S. Fish and Wildlife Service), reported unsubstantiated statistics in the U.S. Department of Agriculture's Yearbook of Agriculture that 256 prairie dogs consume as much grass as a 1,000 pound steer, that 32 prairie dogs consume as much as one sheep, and that these rodents contribute to a 75% decline in rangeland productivity. These fabricated numbers influenced farmers, ranchers and government agencies to nearly decimate prairie dog populations via federally-funded mass poisonings, fumigations, drownings, and shootings (Hoogland, 1995; Graves, 2001).

Although the exact number has recently become a topic of controversy (Vermeire et al, 2004; Forrest, 2005), it has been estimated that as many as five billion prairie dogs (all prairie dog species were included in this number) were alive in the U.S. in the late 1800's (Merriam, 1902). Today, prairie dog abundance is commonly expressed in terms of surface area occupied by their colonies (Miller and Cully, 2001). It is currently estimated that black-tailed prairie dogs occupy less than 1% of their historical U.S. habitat (Miller and Cully, 2001) with the largest decline between 1870 (46,931,892

hectares) to 1998 (256,912 hectares) (Graves, 2001; National Wildlife Federation, 1998). The National Wildlife Federation (NWF, 1998) states that presently about 72 percent of the U.S. black-tailed prairie dog habitat, and all the remaining large complexes of black-tailed prairie dog towns, occur in three states: Montana, South Dakota and Wyoming.

Along with prairie dogs, numerous other prairie-dwelling animals [black-footed ferret (*Mustela nigripes*), bison (*Bison bison*), swift fox (*Vulpes velox*), burrowing owl (*Athene cunicularia*), ferruginous hawk (*Buteo regalis*), and mountain plovers (*Charadrius montanus*)] have also experienced tremendous reductions in numbers. The survival of these and many other species is intertwined with that of the prairie dog. These species prey on prairie dogs, find critical shelter in their burrows and/or benefit from other prairie dog activities that collectively maintain open, herbaceous habitats (Lomolino and Smith, 2001). Miller, et al. (1999) estimated that nine species of animals depend on prairie dogs, 20 species have opportunistic use of prairie dog colonies and 117 other species have life histories that likely benefit from prairie dog activities. A general consensus exists, although not without controversy (Stapp, 1998), that the prairie dog is a keystone species of the Great Plains prairie ecosystem (Kotliar, 2000; Kotliar et al. 1999; Miller et al., 1994, 1999).

Prairie dog populations face a wide range of challenges to their continued survival. Bubonic plague (“Black Death”) is caused by the bacterial species *Yersinia pestis* and vectored by fleas. It is known as sylvatic plague when present in ground squirrels and other wild animals. Most likely originating from Asia, sylvatic plague is speculated to have entered United States ports approximately 100 years ago and has

currently become established in wild rodent populations of the western U.S. (Cully and Williams, 2001). This disease has been documented in all four U.S. prairie dog species for the past 60 years, frequently killing >99% of prairie dogs in infected colonies (Cully and Williams, 2001). Barnes, 1993, has reported that plague is the only infectious disease known to cause extensive die-offs in prairie dogs. The major impacts of plague include local extirpation of colonies, increase in the probability of extinction of entire complexes, reduction of colony size, increase of intercolony distances within colony complexes, increase in distances between colony complexes, increase variance in local population sizes, and reduction in the effectiveness of dispersal in demographic rescue among colonies (Cully and Williams, 2001). Cully and Williams, 2001, have concluded that no evidence exists to suggest that prairie dogs have yet to evolve/develop any resistance to plague.

Prevailing myths and century-old attitudes towards the prairie dog have lead to extensive government-sponsored as well as private rodent warfare programs that have contributed to the marked decrease in prairie dog populations. Although the government-sponsored rodent warfare programs have decreased since the 1970's, negative perceptions of the prairie dog still persist and hence, unfounded efforts to eradicate the animals continue. The wholesale loss of available prairie dog habitat has further compounded the lethal effects of plague and rodent warfare activities to provide an additional cause contributing to the decline of this species.

The dramatic reduction in prairie dogs over their former range has the attention of various governmental agencies and conservation organizations. However, the National Wildlife Federation's (NWF) proposal in 1998 to list the black-tailed prairie dog

as a threatened species was denied even though at least four of the five requirements for listing were met (only one is required for listing under the Endangered Species Act) (Miller and Cully, 2001). Still, prairie dogs remain as “species of concern” in most states in which they range. For this reason, various state wildlife agencies have established management plans to determine the status of prairie dogs in their respective states and to initiate conservation efforts necessary for survival of the species. The management plan for Texas was drafted in early 2004 and includes various goals with objectives and strategies. A study of population genetics of extant colonies will be beneficial to help meet the goals set forth by the state of Texas (TPW, 2004).

Social Structure of the Black-tailed Prairie Dog

A characteristic feature of all prairie dog species is coloniality. Black-tailed prairie dog (*Cynomys ludovicianus*) colonies are organized into family groups, called coteries, which are harem-polygynous units. Coteries typically include a breeding male, two or three adult females, and one or two juveniles and /or yearlings of each gender with a mean coterie size (± sd) of 6.13 ± 3.53 individuals (Hoogland, 1995). However, coterie size and makeup can vary widely, being particularly dependent upon the previous year’s weather and the size of the coterie home territory (Hoogland, 1995). Females tend to remain in their natal coterie territories for their entire lifetimes, while males usually disperse from the coterie after two years. Hence, females of a coterie are likely to be closely related while sexually mature males can be expected to have come from other coterie units of the colony or immigrants from nearby colonies (N = 273 coteries; Hoogland, 1995).

Importance of the Study of Population Genetics

Understanding the population genetics of prairie dog colonies is essential for long-term monitoring of their health and survival. Prairie dogs seldom migrate, and when they do, it is only over short distances, e.g., ≤ 5 km (Garrett and Franklin, 1988). Massive habitat destruction, the effects of plague and animal eradication programs have combined to convert most historical prairie dog populations into isolated groups with few hospitable migration corridors to allow adequate gene flow between populations (Roach et al, 2001). This forces prairie dogs throughout the Great Plains to rely on the existing genetics within these isolated metapopulations. One property of metapopulations is a recurrent pattern of localized extinction and recolonization of individual populations within the extended network (Lidicker and Koenig, 1996). The dynamics of the population genetics of the entire unit depends upon dispersal between the metapopulation subunits. Therefore, given the increased isolation of prairie dog colonies, their current and future genetic diversity, genetic drift, founder effect and bottlenecking are major concerns.

These concerns have lead to the present study, where a molecular-based approach involving microsatellite analyses were used to measure and compare the amount of genetic diversity in selected extant black-tailed prairie dog colonies throughout their current range in Texas. The results from this study provide baseline data which will aid future preservation, conservation and restoration projects involving this and associated animal species.

Population Genetics of Black-tailed Prairie Dog Populations

The earliest studies of black-tailed prairie dog population genetics were performed by Chesser (1983) using allozyme variation at seven variable protein loci. A total of 21 geographical sites within four regions of eastern New Mexico were studied. Data were used to estimate heterozygosity (H_E) as a measure of overall genetic variation, as well as to determine genetic differentiation using Wright's (1965) F-statistics as modified by Nei (1977). Pair-wise genetic similarities between populations were also calculated using the genetic identity measure (I) of Nei (1972). Based on the analysis of F_{ST} (genetic differentiation over subpopulations), Chesser (1983) found significant but moderate differentiation between individual study regions and also populations from within each region. He further detected an excess of homozygous individuals within populations, as measured by Wright's F_{IS}, indicating elevated levels of inbreeding within subpopulations. Surprisingly, Chesser (1983) found greater genetic differentiation between some colonies in the same region than between regions. Collectively, the data indicated that the populations of black-tailed prairie dogs fit a model of differentiation by founder effect, mutation, and genetic drift.

In another study, Dobson et al. (1998) examined genetic variation over ten years in a single South Dakota black-tailed prairie dog population. Mating within the population approached randomness with mates tending to originate from different coteries. Dobson et al., determined allozyme variation at four variable loci and analyzed data using Wright's (1965) F-statistics. They found substantial genetic differentiation between coteries of the population, with 15 to 20% of the genetic variation occurring

among the coteries. A measure of inbreeding (F_{IS}) was negative and low, indicating that inbreeding is not prevalent within this population.

Similarly, Travis et al. (1997) analyzed genetic variation in two populations of Gunnison's prairie dog (*Cynomys gunnisoni*) using minisatellite DNA profiles. Using an F-statistic analog, heterogeneity of the two populations was determined to be similar to black-tailed prairie dog allozyme-based F_{ST} values reported by Chesser (1983).

More recently, molecular-based analyses of highly polymorphic microsatellite or short tandem repeat (STR) loci have been used for detailed studies of population genetics in a variety of animals. Microsatellite loci consist of tandemly repeated sequences of two to six nucleotides. Individual alleles vary by the number of repeats encoded, with individual alleles varying from several to more than 40 copies of the repeat unit. For example, allele designations are written as CA_n, where n is the number of repeats. Thus, CA_{20} indicates an allele of 20 repeat units of the dinucleotide repeat CA (C,G,A, and T are the designations for the bases of the DNA code which include cytosine, guanine, adenine, and thymine). Microsatellites are scattered throughout the genome of higher eukaryotes and are commonly found in non-coding regions of the DNA. Once the sequences of the region flanking a particular microsatellite locus are determined, one can readily analyze the allelic makeup of individuals (and thus populations) at that locus by way of polymerase chain reaction (PCR). The hyperpolymorphic nature of many microsatellite loci is the result of mutations believed to result from the process of slippage during replication. The repetitive nature of these loci stabilizes mispairings between the newly synthesized DNA and the template strand, increasing the likelihood of replication errors that increase or decrease the size of the

STR, with integeric unit changes much more likely to occur. Thus, over time, a given population will experience a general increase in the number of length variants (alleles) at these loci.

In practice, PCR amplified fragments of the different alleles, e.g., CA_{24} and $CA_{26,}$ can be separated as bands based upon their migratory properties on a test electrophoretic gel that can be visualized by a variety of detection techniques. The size variants represent allele variation and are indicative of sequence diversity in the genetic material. At any particular locus, a homozygous individual will exhibit a single band/allele while the heterozygous individual will yield two bands following electrophoretic analysis. Typical vertebrate populations may have as many as five to 15 alleles at polymorphic microsatellite loci. Determination of allelic variation at STR loci allows one to perform the same types of genetic analyses that formerly utilized allozymic data, with the STR data generally providing a more detailed data set than was possible with allozyme studies. Potential applications of microsatellite DNA analyses include individual identification, parentage analyses, relatedness calculations, genetic differentiation between populations or species, and the identification of demographic bottlenecks in species of concern to conservationists.

Demographic bottlenecks (Mayr, 1963) occur when populations experience temporary but severe reductions in population size, where the small number of surviving individuals does not represent a random sample or a complete sampling of the genes in the parental population. For this reason bottlenecks can produce dramatic reductions in a population's gene pool (genetic diversity often measured as H_E). This loss of genetic diversity can reduce the potential of these populations to respond to disease and other

challenges such as both short and long-term environmental variation (Allendorf and Leary, 1986; O'Brien, 1994; Taylor et al., 1994). As a result, reduced genetic diversity is clearly non-adaptive as environments change. Low levels of gene diversity in populations due to bottlenecks, or populations that result from the reintroduction of small, nonrandom samples of the gene pool, e.g. metapopulations, have become a common and important theme in conservation biology. Indeed, low levels of genetic variation have been identified in several threatened or endangered species, including Greater Prairie Chickens (*Tympanuchus cupido*; Bouzat et al., 1998), loggerhead shrikes (*Lanius ludovicianus*; Mundy et al., 1997) and Ethiopian wolves (*Canis simensis*; Gottelli et al., 1994). Thus, the measurement of gene diversity in sample populations designated for recolonization is of extreme importance in conservation biology.

Microsatellite DNA has already proven useful in monitoring gene diversity in a variety of mammalian species designated for conservation and management. This technology has been essential in determining genetic parameters for black bears (*Ursus americanus luteolus*), which are federally listed as threatened in Louisiana and adjacent regions (Boersen et al., 2003; Csiki et al., 2003; Warrilow et al., 2001). Similarly, microsatellite analysis of the Yellowstone grizzly bear population (*Ursus arctos*) identified a slight reduction in genetic diversity due to inbreeding (Miller and Waits, 2003). The impact of bottlenecks on sea otter populations (*Enhydra lutris*) was examined by Larson et al. (2002), who found lower than expected genetic diversity in those populations impacted by fur trade exploitation. Comparable data were collected for fragmented river otter (*Lontra canadensis*) populations with limited dispersal (Blundell et al., 2002), indicating the importance of preserving genetic diversity in this

species. Microsatellite DNA variation has also been used successfully for determining genetic variation and diversity in native, reintroduced and colonizing populations of Rocky Mountain wolves (*Canis lupus occidentalis*) in both Canada and the northern United States (Boyd, et al., 2001; Forbes and Boyd, 1996, 1997). Finally, applications of microsatellite DNA have proven effective in demonstrating that low levels of genetic diversity exist in relic populations of a diverse array of mammals (Castleberry et al., 2002; Reese et al., 2001; Uphyrkina et al., 2002), including red-backed voles (*Clethrionomys gapperi*) in the southern Appalachians, Allegheny woodrats (*Neotoma magister*) and Asian leopards (*Panthera pardus orientalis*).

Applications of microsatellite profiling to prairie dog populations have already verified its usefulness for characterizing the genetic structure and population dynamics of this species. Roach et al. (2001) examined the genetic structure of 13 colonies of black-tailed prairie dog metapopulations in northern Colorado using microsatellite loci. Here, moderate levels of differentiation were observed and levels of inbreeding were low. Of the individuals sampled, 39% were not assignable to the colony from which they were caught, indicating they were immigrants or offspring of immigrants. Furthermore, age of colony was related to genetic similarity, with older colonies being more similar than were younger colonies. These findings emphasize the importance of retaining corridors for dispersal between colonies, allowing not only for genetic exchange between colonies but also more rapid recolonization or supplementing of colonies decimated by plague or other factors. Recently, Haynie et al. (2003) utilized variation at seven microsatellite DNA loci to determine levels of multiple paternity and breeding success in Gunnison's (*Cynomys gunnisoni*) and Utah (*Cynomys parvidens*) prairie

dogs. The application of microsatellite analyses for the determination of genetic properties of populations of prairie dogs has thus repeatedly demonstrated its utility for monitoring genetic diversity of black-tailed prairie dog populations.

CHAPTER 2

MATERIALS AND METHODS

Ethical Use of Animals

The capture and release of live prairie dogs and collection of fresh blood samples was necessary for the completion of this project. **No animals were sacrificed**. All procedures, including trapping, blood collection, and monitoring of condition were in accordance with animal use protocols approved by the Animal Use and Care Committee at the University of North Texas.

Collection of Prairie Dog Whole Blood

A total of 319 whole blood samples were collected from prairie dogs from 16 sites/colonies located throughout the remaining range of the black-tailed prairie dog in Texas (Figure 1 and Table 1). Due to landowner privacy considerations, the geographical positioning coordinates of collection sites within each county are not provided. However, Dallam County is home to a portion of the Rita Blanca National Grasslands, and this site was included in this study. Collections were carried out between April and October of 2005. Sites were chosen in order to provide samples from isolated colonies at the extremes of the present range, as well as from much larger metapopulation clusters (five sites were located in Lubbock County and two in Hemphill County). This allows for the assessment of both total allelic diversity (gene pool) and heterozygosity levels in the entire Texas population.

Samples were collected from an average of 20 prairie dogs at each site by one of two methods. One technique included an FDA-approved capture involved pumping

water and soap into a prairie dog burrow until the prairie dog emerges into the control of an FDA-licensed, prairie dog handler. An alternative method involved the use of 24 x 6 x 6 inch Tomahawk Live Traps® (Tomahawk Live Trap Co., Tomahawk, WI) baited with whole oats. Traps were monitored continuously, using binoculars to determine when animals were captured. Animals were sexed by visual examination. The anus and vaginal groove are in close proximity in females as compared to the anus and penis/scrotal sac in males (Hoogland, 1995). Next, 40 – 700 microliters (µl) of whole blood for microsatellite analyses was collected into Microtainer® Brand tubes (Becton Dickininson Vacutainer Systems, Franklin Lakes, NJ) with the anticoagulant, ethylenediaminetetraacetic acid with potassium [EDTA (K_2)] by clipping a claw on one foot immediately proximal to the distal end of the subunguis. Following blood collection, the clipped claw was dabbed with a liquid-filled Veterinarian's Best® Pet Swab™ (Veterinarian's Best, Inc., Santa Barbara, CA) to reduce pain and bleeding. The captured prairie dogs were maintained in 1 x 0.3 x 0.3 meter cages for a short time to ensure bleeding had stopped. Prairie dogs were then released at their point of capture. A portion of this study was also conducted in conjunction with state- and FDA-approved relocation projects. Individuals from colonies undergoing relocation were first quarantined for two weeks in an FDA-approved facility before blood samples were drawn. These prairie dogs were later released at their new relocation sites.

Long Term Storage of Prairie Dog Whole Blood Samples

The movement of prairie dogs or their blood samples is subject to the interim final rule entitled "African Rodents and Other Animals that May Carry the Monkeypox Virus" (Title 21, Code of Federal Regulations, Section 1240.63). Therefore, following

blood collection, the sealed Microtainer® Brand tubes (Becton Dickininson Vacutainer Systems, Franklin Lakes, NJ) were quickly centrifuged and transported in a secured cooler to the Molecular Biology Laboratory at the University of North Texas in Denton, Texas. Samples were then stored at -20°C in sealed racks in a secure freezer. At the conclusion of this research project, samples were autoclaved prior to disposal in accordance with the Department of Health and Human Services regulations.

DNA Isolation from Prairie Dog Whole Blood Samples

In mammals, chromosomal DNA can be obtained from white blood cells found in whole blood. First, white blood cells are separated from red blood cells, platelets, and plasma. Next, DNA was extracted from the white blood cells via a modified version of the guanidinium (iso)thiocyanate DNA extraction method (GITC) of Hammond et al. (1996). This approach was utilized due to its comparative simplicity and its ability to provide good yields of genomic DNA from relatively small blood samples.

Twenty microliters of whole prairie dog blood was added to 500 µl of the extraction solution (0.5 M guanidinium thiocyanate and 0.1 M EDTA) in a sterile 1.7 ml microcentrifuge tube. A 250 µl aliquot of ice-cold 7.5 M ammonium acetate was then added, the contents of the tube vortexed well, and the solution incubated on ice for 10 min. After a brief centrifugation to precipitate the contents to the bottom of the tube, 500 µl of a 24:1 chloroform: isoamyl alcohol was added and the mixture again vortexed well. After centrifugation at 10,000 rpm for 10 min at room temperature, the upper aqueous phase was transferred to a new, sterile 1.7 ml microfuge tube and the remaining chloroform mixture properly discarded. A second extraction with 500 µl of 24:1 chloroform: isoamyl alcohol as before and the final aqueous phase following

centrifugation was again transferred to a new, sterile 1.7 ml microfuge tube. The DNA was then precipitated by adding 600 µl cold isopropanol, vortexing well, and storage overnight at -20˚C. The precipitated DNA was collected by centrifugation at 13,000 rpm for 20 min at 4˚C in a Heraeus® Fresco™ Microcentrifuge (Thermo Electron Corporation, Milford, MA). The supernatant was removed using a pulled-out Pasteur pipette, leaving a small pellet of DNA. One milliliter of cold 70% ethanol was added to the microfuge tube containing the DNA pellet, which was then gently inverted three times. The washed pellet was again collected by centrifugation at 13,000 rpm for five minutes at 4˚C. The 70% ethanol supernatant was carefully removed using a pulled-out Pasteur pipette, taking care not to disturb the small DNA pellet. Residual ethanol was removed using a Speed Vac™ (Savant Instruments, Inc., Farmingdale, NY) vacuum concentrator for seven min. This final dry pellet was then resuspended in 20 µl of TE Buffer (10 mM Tris/HCl, pH 8.0; 1 mM EDTA) and stored at -20˚C. DNA was quantified using a SmartSpec™ 3000 Spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA).

Primer Preparation

The DNA oligonucleotide primers (Jones, et al., 2005) used to amplify target microsatellite loci were obtained from Bio-Synthesis, Inc. (Lewisville, Texas). Unopened primer tubes were centrifuged to recover any primer residue from the lid using a Savant HSC10K Speedfuge (Savant Instruments, Inc., Farmingdale, NY). Two hundred microliters of sterile double deionized water (ddH_2O) was added to each primer tube and the tube was then vortexed well. Quantification of the primer sample was accomplished using a SmartSpec™ 3000 Spectrophotometer (Bio-Rad Laboratories,

Inc., Hercules, CA). Single-stranded DNA has a maximum absorption at approximately 260 nm, with a mg/ml solution having an absorption of about 30 absorbance units. Scans of each primer sample were taken between 220-320 nanometers (nm). The absorbance of each diluted primer solution was recorded and used to calculate the final amount of ddH2O needed to adjust each primer to a final concentration of 100 µM. A 10 µM working solution was routinely made for each primer.

PCR Amplification

Six loci, three with tetrameric repeat units and three with dimeric repeat units, were ultimately chosen from the original14 microsatellite loci characterized and known to be polymorphic in the black-tailed prairie dog (Jones et al., 2005). PCR was performed using an MJ Research® Peltier Thermal Cycler 200 (Bio-Rad Laboratories, Inc., Hercules, CA) using GeneMate® 96 well PCR plates (ISC BioExpress, Kaysville, UT) and sealed with ThermalSeal™ adhesive sealing films (Excel Scientific, Inc., Wrightwood, CA). Reaction wells contained 20 µl reaction volumes consisting of 0.1 – 2.5 ng of template prairie dog DNA, 0.2 mM deoxyribonucleotides (dNTPs) (New England BioLabs, Ipswich, ME), 0.04 µM γ-^{32}P-end labeled forward primer (see below), 0.1 µM of both non-labeled forward and reverse primers, 0.5 units per reaction *Taq* DNA Polymerase (New England BioLabs, Ipswich, ME), and 1X final concentration of ThermoPol Reaction Buffer (New England Biolabs, Ipswich, ME). PCR cycle conditions were three min at 94°C; followed by 30 cycles of 30 sec at 94°C, one min at T_a°C [annealing temperature, specific for each primer set, see table 2], one min at 72°C; followed by four min at 72°C, and held at 4°C.

Primer Labeling

Forward primers were labeled in a 20 µl reaction containing 2 µM forward primer, 11.1 kilobequerels (KBq) per reaction γ-^{32}P-dATP (specific activity 111 Bq/mmol), 10 units/µl T4 Polynucleotide Kinase (PNK) (New England BioLabs, Ipswich, ME), and 1X final PNK Buffer (New England BioLabs, Ipswich, ME). This mixture was incubated at 37°C for one hr, yielding forward primers carrying $^{32}P0_4$ at their 5' ends.

Optimal Annealing Temperature Determination

The optimal annealing temperature for a given set of primers used in PCR amplification may differ from laboratory to laboratory due to differences in the brand of thermostable DNA polymerase used, the make and/or model of thermacycler, or any of a number of other factors. For this reason the annealing temperatures for the six loci chosen for this project where optimized using an MJ Research® Peltier Thermal Cycler 200 (Bio-Rad Laboratories, Inc., Hercules, CA) and its temperature gradient capability. A total of eight annealing temperatures (45, 46.1, 47.9, 50.5, 54.4, 57.1, 58.9, and 60°C) were evaluated for each of the six loci. Amplified samples were electrophoresed on 8% acrylamide gels (see below) and compared to each other with respect to both quantity and quality of the amplification product. Optimal annealing temperatures for each locus are listed in Table I-2.1.

TABLE I-2.1. Primer sets, annealing temperatures (T_a), allele size ranges, and number of alleles (A) for microsatellite loci used in the genetic analysis of black-tailed prairie dog (*Cynomys ludovicianus*) populations.

Locus*	Primer sequence (5'-3')*	Repeat	T_a	Size range (base pairs)	A
A2	CCATTCTACATCCCAGGAG AGCCAGTATGATTTAGGTGGT	$(AC)_{14}$	55	208-220	10
A111	TCCCACTCTACTTAGCAAAAAT CCTACCTCGTCTTAAAAAATTG	$(GT)_{19}$	55	169-175	10
A115	TGTCCCCCCTAAAAGTAGC CAGAAAATAGCCCAAATGTTC	$(AC)_{15}$	47.5	176-186	7
D1	ACCTTTTGTTTCATTCTCAGC TGCCATAGTTTGCTTTCTTACT	$(TATC)_{10}$	60	178-202	6
D12	TTACCTCCCCACACACAAA TGCCTCACTATTGGACAGC	$(TAGA)_6$	55	192-208	6
D115	CAGGCATCTATGGAAGACAG CTTTGATTGGTGAGTTTTGTG	$(TAGA)_{11}$	57	188-208	8

*Source = NCBI, 2007 and Jones et al., 2005.

Acrylamide Gel Electrophoresis

A mixture of 8 ml 50% Long Ranger® Gel Solution (Cambrex, Inc. East Rutherford, NJ), 10 ml of 5X TBE (formula below), and then brought up to 50 ml with ddH_2O was suction filtered. Next, 0.03 g ammonium persulfate was added and the mixture degassed *in vacuo* for one min. Seven microliters of TEMED (tetramethylethylenediamine) was added, and the mixture was then swirled and poured into a clean 250 ml glass beaker.

A 50 ml syringe was then used to "pour" this solution between two clean 20 cm X 20 cm siliconized glass plates that had been with a 5% dichlorodimethyl silane in heptane solution using latex gloves under the hood. The glass plates were separated by 0.75 mm Delrin® (Dupont™) spacers on all sides (the top spacer was added after the solution was poured) and the gels were allowed to polymerize for a minimum of 1-2 hrs. After polymerization, the top and bottom spacers were removed and the glass was rinsed with ddH_2O. The glass cassette was then dried and loaded into an electrophoretic chamber. TBE buffer (1X; next section) was added to both the top and bottom chambers, air bubbles were removed from both top and bottom gel spaces using a Pasteur pipette, and the gel was pre-electrophoresed for one hr at 250 volts (V).

After pre-electrophoresis, the buffer was removed from the top chamber and the top gel space was dried using a clean paper towel. The space was then filled with one ml Ficoll™ PM400 solution (Amersham Biosciences, Pittsburgh, PA). Five microliters of 5X OG + XC (orange green + xylene cyanol) loading buffer was added to each 20 µl sample. Using a 64 lane rapid loading membrane tray (The Gel Company, San Francisco, CA), 0.8 µl of each sample as well as 0.8 µl of pBr322 cut with *Hin*fI ladder

with loading buffer was added to each well and absorbed onto the teeth of a 64 lane porous membrane comb (2.2 mm spacing, 0.2 mm thick) (The Gel Company, San Francisco, CA). This comb was then place in the Ficoll™ PM400 solution (Amersham Biosciences, Pittsburgh, PA) filled well, contacting the top of the gel. The top buffer chamber was then refilled with 1X TBE and the samples migrated from the comb into the gel when 250 volts (V) was applied to the gel for one min. Power was halted, the comb removed, and the Ficoll™ PM400 (Amersham Biosciences, Pittsburgh, PA) solution was washed from the space with a Pasteur pipette using the buffer in the top chamber. The gels were further electrophoresed at 350 V until the fastest migrating loading dye ran off the bottom of the gel.

The gel was gently removed from the glass plates and placed in a SYBR® Green nucleic acid gel staining solution (Molecular Probes, Inc., Eugene, OR) for 30 min. Gels were observed using a Molecular Imager® GS 800 Calibrated Densitometer (BioRad Bio-Rad Laboratories, Inc., Hercules, CA).

Tris Borate EDTA (5X TBE)

First, 538.9 g Sigma® Trizma® Base (Sigma-Aldrich Co., St. Louis, MO) are added to five L ddH_2O and mixed until dissolved. Next, 275.01 g boric acid is then added and mixed until dissolved. Lastly, 37.2 g disodium ethylenediamine tetraacetate ($Na_2EDTA{\cdot}2H_2O$) is added, the solution was brought up to 10 L with ddH_2O, and then mixed until completely dissolved (approximately 24 hours).

Electrophoretic Analyses of PCR Products

PCR products were electrophoresed at a power level of 35 watts (approximately 1200 volts) until the bromophenol blue loading dye had migrated 33 cm of a 50 cm x 0.25 mm 5.75% Long Ranger® (Cambrex, Inc. East Rutherford, NJ) denaturing gel (formula below) using reference allele sizing ladders constructed from reference profiles of known genotypes.. This power level maintains the gel temperature at a minimum of 50°C. Following electrophoresis the gels were transferred to Ahlstrom® blot paper, grade 238 (ISC BioExpress, Kaysville UT), dried on an SE1160 vacuum gel dryer (Hoefer Inc., San Francisco, CA) for 20 min at 70°C, and exposed to blue sensitive KSB X-ray film (KSR X-Ray, Boca Raton, FL) for approximately 20 hr. Exposed films were developed using a Kodak All Pro 100 automatic developer (Kodak, Rochester, NY).

Long Ranger® Denaturing Gel Solution (5.75%; 2 gels)

First, 42 g urea was dissolved in 37 ml ddH_2O and 20 ml 5X TBE. Next, 11.5 ml 50% Long Ranger® Gel Solution (Cambrex, Inc. East Rutherford, NJ) was added and gently mixed. This solution was vacuum filtered. Then, 0.05 g ammonium persulfate was added *in vacuo* and degassed for one min. Lastly, 70 µl Tetramethylethylene-diamine (TEMED) was gently mixed into the solution and poured into a 250 ml glass beaker. This mixture was immediately poured between the glass plates using a 50 ml syringe.

Determination of Nucleotide Repeat Number

Using results from electrophoretic analyses, specific prairie dogs were selected which were each homozygotic for one of the six microsatellite loci. Single allele

amplicons from each homozygotic locus/animal were isolated and utilized for DNA sequence analyses. The sequences of these individually sequenced amplicons allowed for the determination of the exact number of repeat units in each of these alleles and comparison of other alleles to these reference alleles allowed for the assignment of specific individual repeat numbers to each of the alleles observed at all six study microsatellite loci.

The amplifications of samples from the homozygotic reference prairie dog samples were performed using a GeneAmp® PCR System 2400 (Applied BioSystems, Foster City, CA) in 0.2 ml thin-wall thermal cycler tubes (ISC BioExpress, Kaysville, UT). The 100 µl reaction mixture consisted of 4 µl template DNA, four µl 10µM forward primer (Bio-Synthesis, Inc., Lewisville, Texas), four µl 10 µM reverse primer (Bio-Synthesis, Inc.,Lewisville, Texas), eight µL 2.5 mM dNTP (New England BioLabs, Ipswich, ME), 10 µl 10X Termopol Buffer (New England BioLabs, Ipswich, ME), 0.8 µL *Taq* DNA polymerase (New England BioLabs, Ipswich, ME), and 69.2 µl ddH_2O. PCR conditions were the same as described above for each locus in “PCR Amplification”.

The amplified product was mixed with 25 µL of 1X SYBR® Green (Molecular Probes, Inc., Eugene, OR) + 5X OG/XC (orange green/xylene cyanol) loading buffer and the entire sample was electrophoresed at 60 V on a 3% low melting point agarose gel (12 X 14 cm) in 1X Tris-acetate EDTA (TAE) buffer for approximately one hr. [50X TAE was made by adding 242 g Sigma® Trizma® Base (Sigma-Aldrich Co., St. Louis, MO) to 100 ml 0.5 M EDTA and 57.1 ml acetic acid, mixed until completely dissolved, and then the pH is adjusted to 8.5]. A size reference ladder of pBR322 cut with *Hin*fI was electrophoresed as well for a fragment size reference.

DNA Isolation from Agarose

Using a UV transilluminator (Fotodyne® Inc., Hartland, WI) and the *Hin*fI cut pBR322 size reference ladder, DNA amplicons in the size range of approximately 200 base pairs were cut out of the agar using a sterile razor. This DNA-containing agarose slice was then placed in a sterile 1.7 ml microcentrifuge tube and DNA isolated using the vacuum method of the Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI).

First, 10 µl membrane binding solution was added for every 10 mg of gel slice, vortexed, and then incubated at 65°C until the gel slice was completely dissolved. Next, the dissolved mixture was transferred to a Wizard® minicolumn, incubated at room temperature for one min and a vacuum applied until all liquid had passed through the minicolumn. Then, 700 µl membrane was solution was added and vaccumed through. This last step was repeated using 500 µl. The minicolumn was then transferred to a collection tube and centrifuged at 10,000 rpm for five min. The collection tube was emptied, recentrifuged for one min, and the minicolumn transferred to a clean 1.7 ml microcentrifuge tube. Finally, 50 µl of nuclease-free water was added to the minicolumn, incubated at room temperature for 1 min and centrifuged for one min. The minicolumn was discarded and the 1.7 ml microcentrifuge tube contained the amplicon.

Quantification of Cleaned-up Amplicon

Ten microliters of cleaned-up amplicon solution was then subjected to Electrophoretic analysis on a 2% agarose gel in SB at 160 V for 30 min along with references of known amounts of pBR322 cut with *Hin*fI. Using a UV transilluminator (Fotodyne® Inc., Hartland, WI), quantification of the amplicon was visually estimated by

comparison of the intensity of the bands corresponding to the isolated amplicons to the known reference DNAs.

Ten microliters of amplicon solution (1.0 – 6.0 ng/µl) and 10 µl of either a forward or reverse primer (10 µM) were sent to Lone Star Labs, Inc. (Houston, TX) for nucleotide sequence analysis on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Electropherogram data from each sequenced amplicon was compared to the corresponding locus nucleotide sequence submitted by Jones, et al. (2005) to the National Center for Biotechnology Information (2007).

Statistical Analyses

GENEPOP Version 3.4 (Raymond and Rousset, 1995) was used to calculate observed genotypic frequencies and to test for conformation to Hardy-Weinberg expectations, heterozygote deficiencies, and linkage disequilibrium. Comparisons of observed (H_O) and expected (H_E) heterozygosity frequencies were also made. Linkage disequilibrium tests employed the randomization method of Raymond and Rousset (1995) for all locus pairs. GENEPOP was used to perform pairwise tests of genic differentiation for each population pair as well.

The degree of genetic differentiation over subpopulations was estimated using GENEPOP to calculate Wright's fixation index (F_{ST}) for each of the six polymorphic loci (as well as a combined mean) using a "weighted" analysis of variance (Cockerham, 1973; Weir and Cockerham, 1984). Additionally, GENEPOP was used to measure deviations from Hardy-Weinberg proportions for both Wright's inbreeding coefficient (F_{IS}), which measures reduction in heterozygosity of an individual due to nonrandom mating within its subpopulation, and Wright's overall inbreeding coefficient (F_{IT}), which

measures the reduction in heterozygosity of an individual relative to the total population for each of the six polymorphic loci. A combined mean was also calculated for both.

SAS (ver. 9.3.1) was used to calculate the Spearman rank correlation probabilities for comparisons between pairwise genetic distances and pairwise geographic distances among the subpopulations. Significance was defined as $p \leq 0.05$ for any statistical analysis.

Geographical Information System (GIS)

Figure 1 was produced using ArcGIS 9.2 GIS and mapping software (ArcView®, ArcEditor™, and ArcInfo™) (ESRI, Redlands, CA). ArcGIS was also used to calculate the geographic distances between each of the 16 colonies.

CHAPTER 3

RESULTS AND DISCUSSION

Colony Characterization

The sample group for this study consisted of 319 prairie dogs captured from 16 sites/colonies in 11 northern and western Texas counties (Figure I-3.1). The sampling strategy was to collect no more than one each adult male and female from widely spaced burrows throughout the colony, and by this approach to minimize the collection of closely related animals. The gender of 311 of the 319 individuals captured was determined by external examination and an analysis of this data yielded a final sample group makeup of 154 females and 157 males (Table I-3.1).

Based upon geographical positioning system (GPS) coordinates, the minimum and the maximum pairwise colony distances were 6.6 and 780.5 km, respectively (Table I-3.2). Five of the study colonies have now been completely relocated / eradicated and thus no longer exist (COC, LUBA, LUBC, LUBE, and TAR). Currently, one additional study colony (LUBD) is in the process of being relocated / eradicated.

Figure I-3.1. Texas counties where prairie dogs were collected for microsatellite variation analyses. Details of past and existing prairie dog ranges are also given.

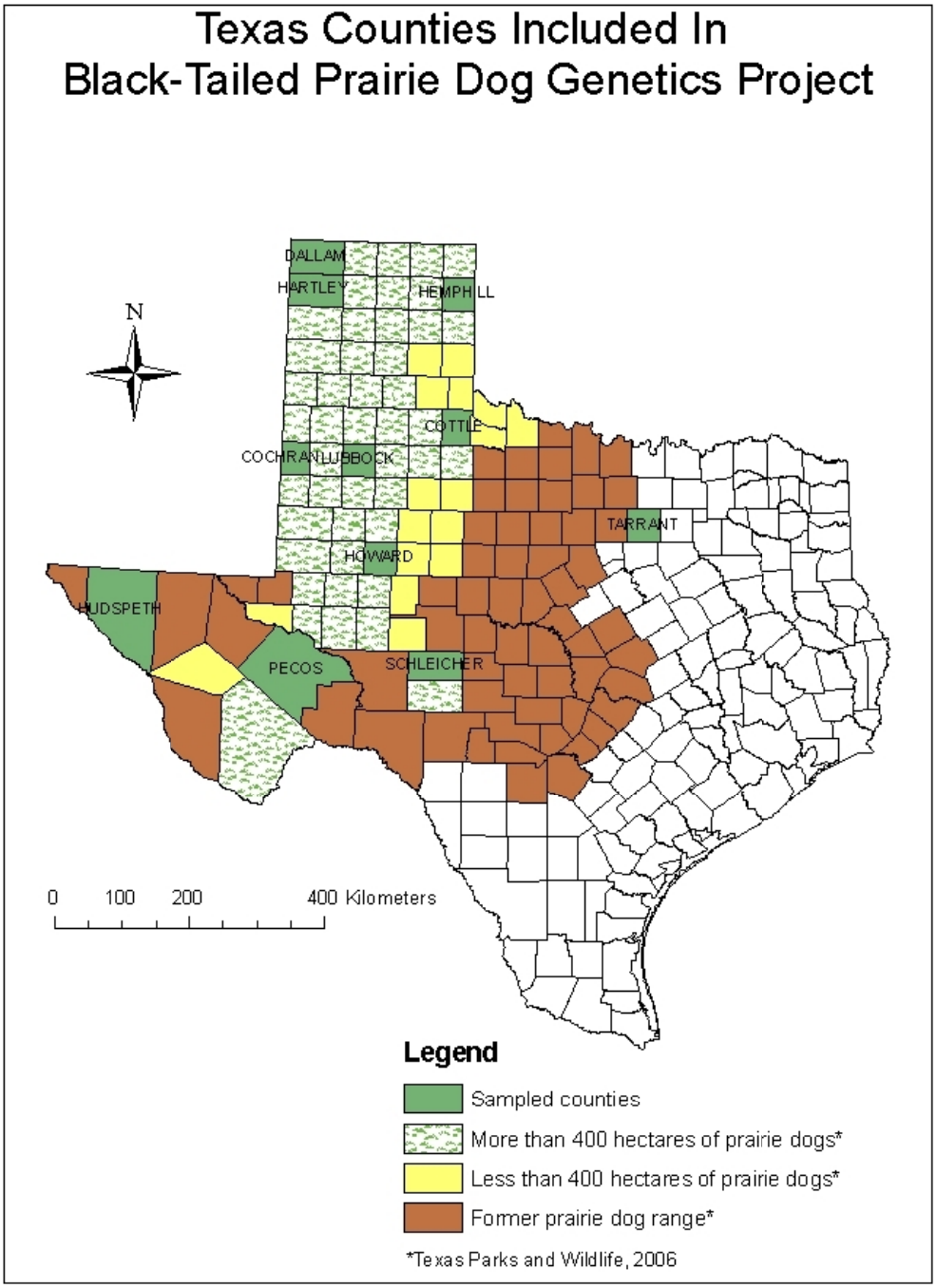

TABLE I-3.1. Colony size, number (*N*), and gender of black- tailed prairie dogs (*Cynomys ludovicianus*) collected at 16 sites in Texas.

County	Site Name	Hectares*	*N*	Males	Females	Unknown Gender
Cochran	COC	101.78	16	10	6	0
Cottle	COT	29.70	22	12	8	2
Dallam	DAL	28.45	23	13	10	0
Hartley	HAR	26.55	20	11	9	0
Hemphill	HEMA	12.99	21	11	9	2
Hemphill	HEMB	3.40	13	11	2	0
Howard	HOW	262.84	22	13	9	0
Hudspeth	HUD	106.51	17	8	9	0
Lubbock	LUBA	ND	21	11	9	1
Lubbock	LUBB	8.74	23	10	12	1
Lubbock	LUBC	250.54	25	10	14	1
Lubbock	LUBD	16.79	19	5	14	0
Lubbock	LUBE	10.16	22	9	13	0
Pecos	PEC	10.60	22	10	11	1
Schleicher	SCH	17.44	14	8	6	0
Tarrant	TAR	4.05	19	5	14	0
Total			319	157	155	8

*Texas Parks and Wildlife, 2006.

TABLE I-3.2. Distances between sampled prairie dog colonies in kilometers.

Site	COC	COT	DAL	HAR	HEMA	HEMB	HOW	HUD	LUBA	LUBB	LUBC	LUBD	LUBE	PEC	SCH
COT	226.3														
DAL	300.4	311.0													
HAR	223.5	255.2	77.3												
HEMA	292.7	172.3	177.5	161.4											
HEMB	298.8	179.3	175.3	162.5	7.3										
HOW	199.6	247.1	473.1	397.4	398.5	405.8									
HUD	348.3	559.5	602.4	535.6	639.1	644.8	377.8								
LUBA	65.8	173.2	320.3	243.7	273.6	280.4	154.9	387.3							
LUBB	78.4	165.5	327.2	251.1	273.6	280.5	146.4	394.1	12.7						
LUBC	63.1	179.6	324.9	248.2	280.3	287.1	151.5	380.6	6.9	16.1					
LUBD	70.4	165.6	316.0	239.7	266.4	273.3	158.0	395.1	7.8	11.9	14.7				
LUBE	77.0	160.3	318.3	242.3	264.8	271.7	155.1	399.8	13.0	9.3	19.3	6.6			
PEC	320.8	459.2	620.9	544.3	591.2	598.2	219.6	230.1	318.5	317.7	312.1	325.4	326.6		
SCH	392.4	381.2	654.0	580.6	551.4	558.6	192.7	490.2	344.1	334.1	341.7	346.0	342.0	268.0	
TAR	515.0	323.3	620.1	574.6	450.4	455.0	402.8	780.5	449.7	437.5	453.5	444.7	438.0	599.1	370.0

Allelic Frequencies

The allelic frequencies for the six microsatellite loci in each population are recorded in Tables 1.4 through 1.9.

The loci may be collectively characterized as having three to four common alleles each and up to four additional alleles occurring at frequencies below 0.10. The total number of alleles at each locus ranged from six at D1 and D12 to 10 at the more polymorphic A2 and A111 loci. Some alleles showed suggestive evidence of regional-dependent frequency patterns and/or founder/bottleneck effects. For example, from these data it is evident that compared to the sample population as a whole, elevated D1 allele frequencies were found in three of the study colonies. The sampled group from COT exhibited a D1 allele frequency of 0.66 for the nine tetranucleotide repeat allele (or the 9 allele), while those at HUD and SCH had frequencies of 0.79 and 0.57, respectively. The remaining 13 collection sites possessed D1 locus allele frequencies that averaged only 0.18 for the 9 tetranucleotide repeat allele.

A comparison of the allele frequencies determined for the six loci reveals differences of up to 300-fold, with as few as one observance of the 22 allele at the A2 locus to as many as 314 observances of the 11 allele at the D115 locus. Some alleles at several loci were also limited to individuals collected from a single site, such as the 21 and 22 alleles at locus A2 and the 10 allele at locus A111 found only at the HEMA site, and allele 19 at locus A115 which was only observed at the DAL site.

TABLE I-3.3. Allele frequencies of A2 Locus with (*n*) = number of alleles.

		Number of dinucleotide repeats per allele									
Site	*N* *	13	14	15	16	17	18	19	20	21	22
COC	16	(0) .000	(8) .250	(0) .000	(8) .250	(6) .188	(4) .125	(5) .156	(1) .031	(0) .000	(0) .000
COT	22	(0) .000	(0) .000	(7) .159	(31) .704	(1) .023	(1) .023	(1) .023	(3) .068	(0) .000	(0) .000
DAL	23	(0) .000	(7) .152	(2) .043	(5) .109	(19) .414	(11) .239	(0) .000	(2) .043	(0) .000	(0) .000
HAR	20	(0) .000	(5) .125	(0) .000	(7) .175	(21) .525	(4) .100	(3) .075	(0) .000	(0) .000	(0) .000
HEMA	21	(0) .000	(3) .071	(0) .000	(1) .024	(8) .190	(4) .095	(11) .262	(0) .000	(14) .334	(1) .024
HEMB	13	(0) .000	(13) .500	(5) .192	(2) .077	(5) .192	(0) .000	(0) .000	(1) .039	(0) .000	(0) .000
HOW	22	(0) .000	(17) .386	(1) .023	(4) .091	(3) .068	(18) .409	(1) .023	(0) .000	(0) .000	(0) .000
HUD	17	(0) .000	(6) .176	(0) .000	(0) .000	(3) .089	(0) .000	(17) .500	(8) .235	(0) .000	(0) .000
LUBA	21	(0) .000	(0) .000	(0) .000	(8) .190	(10) .238	(11) .262	(11) .262	(2) .048	(0) .000	(0) .000
LUBB	23	(9) .196	(10) .218	(0) .000	(7) .152	(11) .239	(5) .109	(2) .043	(2) .043	(0) .000	(0) .000
LUBC	25	(0) .000	(6) .120	(6) .120	(7) .140	(21) .420	(1) .020	(7) .140	(2) .040	(0) .000	(0) .000
LUBD	19	(5) .132	(2) .053	(11) .289	(6) .158	(14) .368	(0) .000	(0) .000	(0) .000	(0) .000	(0) .000
LUBE	22	(0) .000	(6) .136	(1) .023	(7) .159	(25) .568	(0) .000	(5) .114	(0) .000	(0) .000	(0) .000
PEC	22	(0) .000	(0) .000	(0) .000	(0) .000	(31) .705	(0) .000	(13) .295	(0) .000	(0) .000	(0) .000
SCH	14	(0) .000	(0) .000	(5) .179	(5) .179	(16) .571	(0) .000	(2) .071	(0) .000	(0) .000	(0) .000
TAR	19	(0) .000	(11) .289	(14) .369	(13) .342	(0) .000	(0) .000	(0) .000	(0) .000	(0) .000	(0) .000
Total	(638) 319	(14) .021	(94) .155	(52) .087	(111) .172	(194) .299	(59) .086	(78) .123	(21) .034	(14) .021	(1) .002

**N* = number of prairie dogs sampled

TABLE I-3.4. Allele frequencies of A111 Locus with (*n*) = number of alleles.

		Number of dinucleotide repeats per allele									
Site	*N* *	10	11	12	13	14	15	16	17	18	19
COC	16	(0) .000	(0) .000	(0) .000	(2) .063	(7) .219	(6) .187	(17) .531	(0) .000	(0) .000	(0) .000
COT	22	(0) .000	(3) .068	(0) .000	(6) .136	(2) .045	(0) .000	(33) .751	(0) .000	(0) .000	(0) .000
DAL	23	(0) .000	(4) .087	(0) .000	(0) .000	(19) .413	(0) .000	(19) .413	(4) .087	(0) .000	(0) .000
HAR	20	(0) .000	(0) .000	(0) .000	(2) .050	(12) .300	(0) .000	(16) .400	(10) .250	(0) .000	(0) .000
HEMA	21	(2) .048	(1) .024	(0) .000	(0) .000	(15) .357	(3) .071	(21) .500	(0) .000	(0) .000	(0) .000
HEMB	13	(0) .000	(0) .000	(0) .000	(0) .000	(1) .038	(8) .308	(14) .539	(0) .000	(1) .038	(2) .077
HOW	22	(0) .000	(10) .227	(0) .000	(0) .000	(0) .000	(16) .364	(18) .409	(0) .000	(0) .000	(0) .000
HUD	17	(0) .000	(0) .000	(0) .000	(0) .000	(0) .000	(1) .029	(32) .942	(1) .029	(0) .000	(0) .000
LUBA	21	(0) .000	(10) .238	(0) .000	(0) .000	(0) .000	(20) .476	(11) .262	(1) .024	(0) .000	(0) .000
LUBB	23	(0) .000	(0) .000	(0) .000	(0) .000	(21) .456	(8) .174	(16) .348	(1) .022	(0) .000	(0) .000
LUBC	25	(0) .000	(1) .020	(0) .000	(1) .020	(17) .340	(11) .220	(17) .340	(3) .060	(0) .000	(0) .000
LUBD	19	(0) .000	(9) .237	(0) .000	(2) .053	(0) .000	(13) .342	(12) .315	(2) .053	(0) .000	(0) .000
LUBE	22	(0) .000	(13) .296	(0) .000	(0) .000	(2) .045	(12) .273	(17) .386	(0) .000	(0) .000	(0) .000
PEC	22	(0) .000	(0) .000	(9) .205	(0) .000	(8) .181	(7) .159	(11) .250	(0) .000	(0) .000	(9) .205
SCH	14	(0) .000	(0) .000	(2) .071	(0) .000	(4) .143	(7) .250	(11) .393	(4) .143	(0) .000	(0) .000
TAR	19	(0) .000	(15) .395	(0) .000	(0) .000	(23) .605	(0) .000	(0) .000	(0) .000	(0) .000	(0) .000
Total	(638) 319	(2) .003	(66) .100	(11) .017	(13) .020	(131) .196	(112) .178	(265) .424	(26) .042	(1) .002	(11) .018

**N* = number of praire dogs sampled

TABLE I-3.5. Allele frequencies of A115 Locus with (*n*) = number of alleles.

		Number of dinucleotide repeats per allele						
Site	*N* *	13	14	15	16	17	18	19
COC	16	(10) .313	(1) .031	(9) .281	(5) .156	(4) .125	(3) .094	(0) .000
COT	22	(2) .045	(10) .227	(0) .000	(32) .728	(0) .000	(0) .000	(0) .000
DAL	23	(12) .261	(18) .392	(0) .000	(2) .043	(10) .217	(3) .065	(1) .022
HAR	20	(13) .325	(6) .150	(4) .100	(3) .075	(5) .125	(9) .225	(0) .000
HEMA	21	(17) .405	(8) .190	(6) .143	(6) .143	(5) .119	(0) .000	(0) .000
HEMB	13	(4) .154	(7) .269	(8) .308	(2) .077	(5) .192	(0) .000	(0) .000
HOW	22	(7) .159	(23) .523	(3) .068	(10) .227	(1) .023	(0) .000	(0) .000
HUD	17	(7) .206	(2) .059	(24) .706	(0) .000	(1) .029	(0) .000	(0) .000
LUBA	21	(26) .619	(1) .024	(9) .214	(6) .143	(0) .000	(0) .000	(0) .000
LUBB	22	(31) .704	(4) .091	(0) .000	(9) .205	(0) .000	(0) .000	(0) .000
LUBC	25	(17) .340	(13) .260	(5) .100	(6) .120	(9) .180	(0) .000	(0) .000
LUBD	19	(21) .552	(4) .105	(0) .000	(8) .211	(5) .132	(0) .000	(0) .000
LUBE	22	(16) .364	(6) .136	(0) .000	(18) .409	(4) .091	(0) .000	(0) .000
PEC	22	(12) .273	(6) .136	(1) .023	(9) .205	(16) .363	(0) .000	(0) .000
SCH	14	(15) .536	(8) .286	(3) .107	(2) .071	(0) .000	(0) .000	(0) .000
TAR	18	(12) .334	(0) .000	(1) .027	(23) .639	(0) .000	(0) .000	(0) .000
Total	(634) 317	(222) .349	(117) .180	(73) .130	(141) .216	(65) .100	(15) .024	(1) .001

**N* = number of prairie dogs sampled

TABLE I-3.6. Allele frequencies of D1 Locus with (*n*) = number of alleles.

		Number of tetranucleotide repeats per allele					
Site	*N* *	7	8	9	10	11	12
COC	16	(1) .031	(0) .000	(4) .125	(17) .531	(10) .313	(0) .000
COT	22	(0) .000	(0) .000	(29) .659	(8) .182	(7) .159	(0) .000
DAL	23	(1) .022	(12) .261	(10) .217	(10) .217	(10) .217	(3) .066
HAR	20	(2) .050	(4) .100	(8) .200	(18) .450	(8) .200	(0) .000
HEMA	21	(0) .000	(7) .167	(2) .048	(20) .476	(10) .238	(3) .071
HEMB	13	(0) .000	(6) .231	(9) .346	(11) .423	(0) .000	(0) .000
HOW	22	(0) .000	(0) .000	(16) .364	(21) .477	(6) .136	(1) .023
HUD	17	(0) .000	(0) .000	(27) .795	(5) .147	(1) .029	(1) .029
LUBA	21	(2) .048	(1) .024	(10) .238	(22) .523	(5) .119	(2) .048
LUBB	23	(0) .000	(0) .000	(10) .217	(34) .739	(2) .044	(0) .000
LUBC	25	(3) .060	(0) .000	(7) .140	(32) .640	(0) .000	(8) .160
LUBD	19	(0) .000	(5) .132	(2) .053	(20) .526	(11) .289	(0) .000
LUBE	22	(1) .023	(2) .046	(3) .068	(24) .545	(14) .318	(0) .000
PEC	22	(0) .000	(0) .000	(1) .023	(28) .636	(15) .341	(0) .000
SCH	14	(0) .000	(1) .036	(16) .571	(6) .214	(4) .143	(1) .036
TAR	19	(0) .000	(1) .026	(13) .342	(22) .579	(2) .053	(0) .000
Total	(638) 319	(10) .015	(39) .064	(167) .275	(298) .457	(105) .162	(19) .027

**N* = number of prairie dogs sampled

TABLE I-3.7. Allele frequencies of D12 Locus with (*n*) = number of alleles.

		Number of tetranucleotide repeats per allele					
Site	*N* *	5	6	7	8	9	10
COC	16	(0) .000	(8) .250	(3) .094	(16) .500	(4) .125	(1) .031
COT	22	(0) .000	(0) .000	(0) .000	(17) .386	(7) .159	(20) .455
DAL	23	(2) .044	(14) .304	(4) .087	(18) .391	(3) .065	(5) .109
HAR	20	(0) .000	(8) .200	(6) .150	(20) .500	(4) .100	(2) .050
HEMA	21	(0) .000	(20) .476	(0) .000	(19) .453	(3) .071	(0) .000
HEMB	13	(0) .000	(19) .731	(0) .000	(6) .231	(1) .038	(0) .000
HOW	22	(0) .000	(8) .182	(4) .091	(27) .614	(2) .045	(3) .068
HUD	17	(0) .000	(2) .059	(2) .059	(24) .706	(5) .147	(1) .029
LUBA	21	(0) .000	(9) .214	(0) .000	(15) .357	(1) .024	(17) .405
LUBB	23	(0) .000	(4) .087	(1) .022	(37) .805	(2) .043	(2) .043
LUBC	25	(0) .000	(14) .280	(2) .040	(28) .560	(4) .080	(2) .040
LUBD	19	(0) .000	(10) .263	(0) .000	(23) .605	(0) .000	(5) .132
LUBE	22	(0) .000	(20) .455	(0) .000	(15) .341	(7) .159	(2) .045
PEC	22	(0) .000	(6) .136	(4) .091	(19) .432	(15) .341	(0) .000
SCH	14	(0) .000	(9) .321	(10) .357	(1) .036	(8) .286	(0) .000
TAR	19	(0) .000	(0) .000	(0) .000	(2) .053	(25) .658	(11) .289
Total	(638) 319	(2) .003	(151) .247	(36) .062	(287) .436	(91) .146	(71) .106

**N* = number prairie dogs sampled

TABLE I-3.8. Allele frequencies of D115 Locus with (*n*) = number of alleles.

		Number of tetranucleotide repeats per allele							
Site	*N* *	7	8	9	10	11	12	13	14
COC	16	(4) .125	(0) .000	(1) .031	(6) .188	(21) .656	(0) .000	(0) .000	(0) .000
COT	22	(0) .000	(0) .000	(0) .000	(28) .636	(16) .364	(0) .000	(0) .000	(0) .000
DAL	23	(1) .022	(0) .000	(3) .065	(26) .566	(14) .304	(0) .000	(2) .043	(0) .000
HAR	20	(0) .000	(0) .000	(4) .100	(17) .425	(14) .350	(5) .125	(0) .000	(0) .000
HEMA	21	(1) .024	(1) .024	(1) .024	(12) .286	(18) .428	(3) .071	(6) .143	(0) .000
HEMB	13	(0) .000	(1) .038	(2) .077	(7) .269	(8) .308	(6) .231	(2) .077	(0) .000
HOW	22	(2) .045	(0) .000	(0) .000	(2) .045	(20) .455	(20) .455	(0) .000	(0) .000
HUD	17	(0) .000	(0) .000	(0) .000	(0) .000	(31) .912	(2) .059	(1) .029	(0) .000
LUBA	21	(6) .143	(0) .000	(2) .048	(0) .000	(23) .547	(11) .262	(0) .000	(0) .000
LUBB	23	(9) .196	(0) .000	(11) .239	(14) .304	(11) .239	(0) .000	(0) .000	(1) .022
LUBC	25	(0) .000	(0) .000	(1) .020	(12) .240	(29) .580	(3) .060	(4) .080	(1) .020
LUBD	19	(2) .053	(2) .053	(0) .000	(2) .053	(29) .762	(0) .000	(3) .079	(0) .000
LUBE	22	(0) .000	(0) .000	(0) .000	(2) .045	(26) .592	(3) .068	(13) .295	(0) .000
PEC	22	(2) .045	(5) .114	(0) .000	(0) .000	(32) .727	(5) .114	(0) .000	(0) .000
SCH	14	(2) .071	(0) .000	(0) .000	(2) .071	(18) .644	(6) .214	(0) .000	(0) .000
TAR	19	(34) .895	(0) .000	(0) .000	(0) .000	(4) .105	(0) .000	(0) .000	(0) .000
Total	(638) 319	(63) .101	(9) .014	(25) .038	(130) .196	(314) .497	(64) .104	(31) .047	(2) .003

**N* = number of prairie dogs sampled

Individuals collected from TAR showed this population to have a frequency of the D115 seven tetranucleotide repeat allele of 0.895. This allele had a frequency of no more than 0.20 at any other sampling location, making the results from this locus consistent with the TAR colony having undergone a severe genetic bottleneck in its past. Additional evidence of a possible bottleneck in this colony's past is observed at the remaining five loci. Collectively, the allelic frequency data show that in the TAR population, a maximum of two alleles account for more than 92% of the total for five of the six study loci and 71% at the sixth loci (A2). TAR also had the lowest average number of alleles per locus (2.83, Table I-3.9). These allele frequencies are not surprising given the recent history of this colony. According to the landowner of the TAR colony, this site held more than 100 prairie dogs prior to this study. A few years ago, all but four or five were relocated to a new site, and the remaining four to five prairie dogs then became the founding prairie dogs of the 19 count TAR colony. As part of this study these animals were captured, had blood drawn, and were then also relocated from the original TAR site. Thus, a bottleneck did indeed occur. Also, at the time of collection the nearest prairie dog colony to the TAR colony was more than 50 km away. Hence, the occurrence of natural migration into the colony was highly unlikely. However, the landowner also witnessed people releasing their domesticated prairie dog pets onto the land without permission. If one or more of these pets survived it may have actually increased the genetic diversity at this location, and may be the source of one or more of the low frequency alleles at each study locus.

TABLE I-3.9. Combined A2, A111, A115, D1, D12, and D115 loci population statistics for 16 black-tailed prairie dog (*Cynomys ludovicianus*) populations.

Site	*N**	Expected Heterozygosity	Observed Heterozygosity	Average alleles/locus
COC	16	0.69	0.68	4.83
COT	22	0.49	0.48	3.50
DAL	23	0.72	0.64	5.50
HAR	20	0.72	0.68	4.83
HEMA	21	0.70	0.61	5.33
HEMB	13	0.67	0.69	4.50
HOW	22	0.64	0.54	4.50
HUD	17	0.39	0.33	3.83
LUBA	21	0.66	0.72	4.50
LUBB	23	0.58	0.61	4.50
LUBC	25	0.67	0.67	5.50
LUBD	19	0.62	0.57	4.33
LUBE	22	0.64	0.68	4.33
PEC	22	0.60	0.64	3.83
SCH	14	0.65	0.62	4.33
TAR	19	0.49	0.54	2.83

**N* = sample size

Two years prior to sampling, the colony from Schleicher County (SCH) had been large and seemingly healthy, and was used for collection of juvenile prairie dogs for sale in the pet industry. At the time of sample collection for this study, the colony was dramatically reduced in overall numbers and the remaining prairie dogs were scattered into small groups. It is suspected that this colony had been infected with plague within the intervening two years. As a result of this major population decline, the number of animals captured and sampled was small (*N* = 14). Once again, the allelic frequency data show that in the surviving SCH population, a maximum of two alleles account for

more than 75% of the total for five of the six study loci and 64% at the sixth locus (A111). Not surprisingly, a deviation occurred from the HW equilibrium test (P = 0.05, Table I-3.10). These results are again consistent and supportive of a recent bottleneck.

Population Genetic Structure

As mentioned earlier, the number of identified alleles per locus for the six microsatellite loci characterized ranged from six (D1 and D12) to ten (A2 and A111) in the 319-member study population (Table I-2.1). The mean observed heterozygosity (H_O) for the six loci for individual colonies ranged from 0.33 (HUD) to 0.72 (LUBA) (Table I-3.9). Several of the mean expected heterozygosity (H_E) and H_O values of sampled colonies, e.g., HAR, HEMA, LUBD show H_O values lower than expected. This type of finding is consistent with small genetically isolated colonies that have been reduced in size to levels where genetic drift can be expected to impact allele frequencies. The average number of alleles per locus found at the 16 sampled colonies (Table I-3.9) ranged from 2.83 (TAR) to 5.5 (DAL and LUBC).

The most comprehensive measure of population substructure is F_{ST} (Wright, 1951, 1965). F_{ST} is a measure of the amount of genetic differentiation among subpopulations (colonies). F_{ST} values range from 0 (no genetic divergence between populations) to 1 (extreme genetic subdivision). F_{ST} values up to 0.05 (5%) indicate negligible genetic differentiation whereas >0.25 (25%) means great genetic differentiation within the population analyzed (Dorak, 2007; Cavalli-Sforza, et al., 1994). The average value of F_{ST} for human populations with a large number of DNA polymorphisms is 0.139 and 0.119 for non-DNA polymorphisms (Dorak, 2007; Cavalli-Sforza, et al., 1994).

TABLE I-3.10. Hardy-Weinberg probabilities for 16 black-tailed prairie dog (*Cynomys ludovicianus*) populations.

Site	Locus A2	Locus A111	Locus A115	Locus D1	Locus D12	Locus D115	Combined
COC	0.474	0.397	0.714	0.651	1.000	0.289	0.833
COT	0.144	0.212	1.000	0.497	0.043	0.650	0.214
DAL	0.511	0.603	0.113	0.276	0.108	0.878	0.300
HAR	0.115	0.267	0.246	<0.001	0.307	0.423	0.002
HEMA	0.245	0.830	0.022	0.003	0.284	0.011	0.001
HEMB	1.000	0.183	0.341	0.819	0.642	0.968	0.864
HOW	0.933	0.507	0.589	0.093	0.045	0.281	0.138
HUD	0.214	1.000	1.000	0.117	0.125	0.091	0.177
LUBA	0.870	0.323	0.860	0.093	0.464	0.036	0.201
LUBB	0.039	0.715	0.820	0.569	0.143	0.089	0.134
LUBC	0.320	0.181	0.019	0.324	0.179	0.424	0.050
LUBD	0.569	0.927	0.446	0.009	0.147	0.020	0.022
LUBE	0.584	0.598	1.000	0.564	0.749	0.406	0.934
PEC	0.617	0.190	0.021	0.782	0.183	0.503	0.139
SCH	1.000	0.688	0.178	0.047	0.007	0.695	0.050
TAR	0.532	0.147	0.565	0.885	0.042	1.000	0.381
Total	0.538	0.610	0.128	<0.000	0.002	0.037	<0.000

The average F_{ST} among the 16 Texas populations in this project is approximately 16.4% (Table I-3.11). This value is similar to 11.8% from a Colorado metapopulation study of black-tailed prairie dogs (Roach et al., 2001) and 10.3% for an allozyme study of New Mexico subpopulations (Chesser, 1983). This slight increase in genetic subdivision in the Texas populations relative to the Colorado and New Mexico studies may be due in part to having increased geographical distances between study colonies.

The reduction in heterozygosity due to nonrandom mating within its subpopulation is measured by Wright's inbreeding coefficient (F_{IS}). The value of F_{IS} ranges from -1 to +1. A negative value is indicative of an excess in heterozygotes while a positive value is indicative of a heterozygote deficiency. The F_{IS} among the 16 Texas populations in this project had a mean of 0.025 (Table I-3.11) when data for all six polymorphic loci are combined. This value is slightly higher than that of Roach et al. (2001) average value of F_{IS} = 0.017. Chesser's (1983) value was more than an order of magnitude higher (F_{IS} = 0.330). However, this latter value is magnified since Chesser sampled and compared coteries (closely related familial groups) as well as subpopulations.

Wright's overall inbreeding coefficient (F_{IT}), which measures the reduction in heterozygosity of an individual relative to the total population, was much higher (F_{IT} = 0.185, Table I-3.11) when all six polymorphic loci were included. The F_{IT} values for Roach (2001) and Chesser's (1983) results were similar to the F_{IS} values with F_{IT} = 0.133 and 0.404, respectively.

TABLE I-3.11. F-statistics for each microsatellite locus for 16 black-tailed prairie dog (*Cynomys ludovicianus*) populations.

Locus	F_{IS}	F_{IT}	F_{ST}
A2	-0.055	0.124	0.169
A111	-0.048	0.118	0.158
A115	-0.018	0.145	0.160
D1	0.191	0.294	0.127
D12	0.086	0.227	0.155
D115	0.010	0.224	0.216
Combined	0.025	0.185	0.164

Evaluations of the combined data from the six microsatellite loci reveal an overall combined probability of $P < 0.0001$, with 31% (5 out 16) of the colonies (HAR, HEMA, LUBC, LUBD, AND SCH) deviating from the Hardy-Weinberg (HW) equilibrium at $\alpha \leq 0.05$ (Table I-3.10). Two of the five colonies (HAR and HEMA) had probabilities of <0.001. Additionally, five different colonies also were not in HW equilibrium at ≤ 0.05 for at least one of the study loci. These loci include LUBB at A2, PEC at A115, and COT, HOW and TAR at D12. Additional deviations from HW equilibrium at specific loci are indicated by observed heterozygote deficiencies for COT at A2 and DAL at D1 and D12 (Table I-3.12).

Failure to meet HW expectations can result from a number of factors, and there may or may not be any strong evidence to suggest which factor or factors are responsible for the deviation, e.g., sampling error or null alleles (explained in the next

paragraph). Every possible effort was made to sample colonies throughout the existing Texas black-tailed prairie dog range and to obtain a representative random sample from each. However, sampling error is always a possibility in studies such as this, especially when dealing with alleles that have low frequencies.

TABLE I-3.12. Probability values for heterozygote deficits using Hardy-Weinberg test for 16 black-tailed prairie dog (*Cynomys ludovicianus*) populations.

Site	Locus A2	Locus A111	Locus A115	Locus D1	Locus D12	Locus D115
COC	0.516	0.336	0.536	0.413	0.775	0.451
COT	0.020	0.114	0.739	0.410	0.292	0.888
DAL	0.970	0.879	0.064	0.005	0.016	0.287
HAR	0.310	0.958	0.161	<0.000	0.735	0.616
HEMA	0.608	0.574	0.527	0.003	0.413	0.244
HEMB	0.976	0.153	0.952	0.194	1.000	0.755
HOW	0.740	0.165	0.160	0.122	0.090	0.148
HUD	0.268	1.000	0.550	0.078	0.042	0.091
LUBA	0.409	0.974	0.946	0.093	0.890	0.072
LUBB	0.868	0.784	0.687	0.267	0.095	0.931
LUBC	0.705	0.955	0.615	0.199	0.041	0.533
LUBD	0.538	0.836	0.710	0.011	0.160	0.166
LUBE	0.944	0.355	0.877	0.328	0.528	0.985
PEC	0.922	0.202	0.903	0.816	0.775	0.618
SCH	0.896	0.511	0.962	0.044	0.192	0.407
TAR	0.929	0.992	0.250	0.753	0.024	1.000

Null alleles are alleles that go undetected by the protocol, and when dealing with microsatellite loci are commonly due to mutations at one or more PCR primer binding sites in microsatellite loci. Although we cannot rule out the occurrence of such alleles,

the observation that all of the animals were successfully genotyped indicates that any null alleles (if present), occurred at frequencies too low to ever be homozygous for null alleles (carry two identical null alleles or a null allele matched with a second different null allele). Finally, given the potential for unstable populations of prairie dogs that have been impacted by human perturbations or plague, it would not be surprising for some colonies to be in varying states of recovery or decline and thus fail to meet one or more prerequisites for maintaining HW equilibrium.

Genotypic disequilibrium analyses showed that neither locus pairs A2 and D1 ($P < 0.0001$) nor locus pairs D12 and D115 ($P = 0.034$) genotypes vary completely independent of each other (Table I-3.13). These results suggest that these loci may be linked. Although no evidence of linkage was reported in the original paper describing the isolation of the loci (Jones, et al., 2005), data reported here represents a much more in depth study and thus makes such analyses / determinations possible.

Pairwise comparisons of genic differentiation for each population pair ranged from significantly different ($P < 0.01$) to highly significantly different ($P < 0.0001$) for all pairs except the comparison between HAR and DAL, located 77.3 km apart in adjacent panhandle counties. Each of these colonies is surrounded by more than 6069 hectares of occupied prairie dog land within a 16-km radius situated in the most densely prairie dog occupied land in the state (TPW, 2006). The low *P*-values for the remaining pairwise comparisons is indicative that the majority of the study colonies are becoming isolated to the point where there is insufficient intercolony migration to prevent them from becoming independent genetic units.

TABLE I-3.13. Genotypic disequilibrium test. Probabilities (*P*-value) are given for each locus pair across 16 black-tailed prairie dog (*Cynomys ludovicianus*) populations.

Locus Pair	Chi2[Ŧ]	*P*-value
A2 & A111	28.51	0.644
A2 & A115	43.12	0.091
A2 & D1	91.48	<0.000
A2 & D12	19.69	0.956
A2 & D115	45.14	0.062
A111 & A115	39.37	0.173
A111 & D1	45.61	0.056
A111 & D12	43.15	0.090
A111 & D115	43.21	0.089
A115 & D1	45.25	0.060
A115 & D12	30.57	0.539
A115 & D115	40.42	0.146
D1 & D12	24.85	0.812
D1 & D115	32.39	0.448
D12 & D115	48.08	0.034

[Ŧ]Chi Square value

*degrees of freedom = 32 for each comparison

Further support of insufficient migration among colonies is found in the comparison of pairwise F_{ST} values (Table I-3.14) with pairwise geographical distance (Table I-3.2). Correlation tests were performed among the 120 pairwise comparisons using SAS program. A highly significant correlation ($r_s = 0.51$, $P < 0.0001$) was seen between F_{ST} values and geographic distance (Spearman rank correlation).

A significant correlation did not, however, exist among colonies which were located in closer proximity. For example, five subpopulations were sampled in Lubbock County with distances of 6.6 to 19.3 km apart. Using only these five colonies, results showed no correlation when F_{ST} and geographic distances were compared ($P = 0.907$, Spearman rank correlation). The combined F_{ST} value for the six loci from the five Lubbock County sites was 8.6%, nearly half the value of the 16 sites combined.

TABLE I-3.14. F_{ST} values measuring genetic distance between colonies.

Site	COC	COT	DAL	HAR	HEMA	HEMB	HOW	HUD	LUBA	LUBB	LUBC	LUBD	LUBE	PEC	SCH
COT	0.209														
DAL	0.078	0.198													
HAR	0.036	0.206	0.017												
HEMA	0.032	0.245	0.051	0.046											
HEMB	0.088	0.266	0.094	0.102	0.082										
HOW	0.092	0.242	0.126	0.119	0.123	0.103									
HUD	0.182	0.348	0.281	0.249	0.239	0.269	0.254								
LUBA	0.072	0.263	0.152	0.113	0.105	0.149	0.101	0.255							
LUBB	0.087	0.282	0.120	0.077	0.098	0.190	0.156	0.328	0.132						
LUBC	0.028	0.238	0.061	0.029	0.036	0.092	0.099	0.236	0.085	0.067					
LUBD	0.052	0.271	0.125	0.091	0.085	0.145	0.125	0.287	0.064	0.118	0.049				
LUBE	0.060	0.258	0.115	0.084	0.070	0.117	0.126	0.298	0.087	0.154	0.056	0.037			
PEC	0.085	0.332	0.146	0.094	0.110	0.199	0.182	0.308	0.139	0.180	0.068	0.092	0.071		
SCH	0.104	0.261	0.111	0.086	0.122	0.124	0.151	0.255	0.111	0.200	0.096	0.119	0.104	0.128	
TAR	0.278	0.354	0.294	0.289	0.298	0.341	0.320	0.506	0.284	0.281	0.285	0.317	0.296	0.335	0.311

CHAPTER 4

SUMMARY

The concern for the long term “genetic health” of natural populations that have undergone demographic bottlenecks due to loss of habitat and habitat fragmentation has increased in recent years. In Texas, the black-tailed prairie dog has experienced dramatic declines in abundance over much of its historical range, and many populations occur as relicts. Attempts are now underway to establish preserves and to reintroduce populations into suitable habitats formerly occupied by prairie dogs. Based upon experience with prairie dogs and other species, these initial attempts can be expected to have varying degrees of success and will require many years of effort to meet their ultimate goals. The more immediate goals of the Texas Black-Tailed Prairie Dog Conservation and Management Plan (TPW, 2004) are to monitor genetic diversity and to determine the role it might play in the viability and stability of black-tailed prairie dog populations. Achieving these goals will be vital to the success of any long term management plan. It is imperative that the collection of initiated genetic data in this study be continued to better characterize and monitor gene diversity in existing long-established colonies, and to compare these populations to newly colonized and reintroduced prairie dog towns. Such long term comparisons will allow the effects of repeated localized extinctions and recolonizations on metapopulation structure to be determined.

The primary objective of this study was to produce multiple locus genetic profiles of black-tailed prairie dogs (*Cynomys ludovicianus*) collected from colonies throughout the existing range of prairie dogs in the state of Texas and to use this information to

establish a genetic diversity baseline necessary for continued monitoring of the genetic health of these populations. Our initial assessment of prairie dogs from 16 Texas sites has revealed that the existing populations have sufficient variation at the six microsatellite loci characterized to support the long term usefulness of this approach as a primary genetic tool in conservation and preservation of this species.

An evaluation of the data from the 319 prairie dogs in this Texas study reveals regional-dependent frequency patterns as well as support for founder/bottleneck effects for several of the 16 sites. Among the 6 to 10 alleles per locus, only 3 to 4 common alleles are represented among the 6 microsatellite loci, with an average of 2.83 to 5.5 alleles per locus. Observed heterozygosity values range from 0.33 to 0.72, and 31% of the sites deviated from the Hardy-Weinberg equilibrium.

Even though the state population as a whole may appear genetically diverse, considerable genetic divergence has already occurred among the subpopulations (F_{ST} = 0.164) that collectively make up the statewide population. Genic differentiation supports the hypothesis that allelic distribution is highly significantly different ($P < 0.0001$) across the subpopulations. Additionally, pairwise genetic distance correlated against pairwise geographical distance resulted in a highly significant probability ($P < 0.001$) indicating isolation by distance.

As noted earlier, microsatellite loci findings described in this study represent the preliminary report for an ongoing study of black-tailed prairie dog genetics. Prairie dogs from six (COC, LUBA, LUBC, LUBD, LUBE, and TAR) of the original 16 sites have been relocated/exterminated or were in the process of being relocated. Results indicated the following colonies (COT, DAL, HOW, and HUD) are of sufficient size and possess

ample genetic diversity to be characterized as candidate foundation populations for future preservation efforts. The proximity of small colonies (less than 20 hectares) such as HEMB, LUBB, and PEC, to other colonies should be examined to determine if they are isolated or part of a metapopulation. Colonies (HAR, HEMA, and SCH) with low genetic diversity would be ideal candidates for supplementation with properly chosen individuals. Alternatively, these colonies could be relocated and/or blended with other similar but genetically distinct colonies that would also benefit from the resulting influx of genetic diversity. Prairie dog colony size and available genetic diversity will be two of many issues that will need to be assessed as part of conservation efforts for the prairie dog and also for related ecologically-dependent species such as the black-footed ferret.

PART II:

ESTABLISHING HEMATOLOGY AND BLOOD CHEMISTRY BASELINES FOR PET BLACK-TAILED PRAIRIE DOGS (*Cynomys ludovicianus*) AND COMPARING THIS BASELINE WITH A PREVIOUSLY ESTABLISHED BASELINE FOR WILD BLACK-TAILED PRAIRIE DOGS

CHAPTER 1

INTRODUCTION

The black-tailed prairie dog (*Cynomys ludovicianus*) is one of five prairie dog species found in North America and the only species of the five that is legally allowed to be owned as a domesticated pet. These primarily herbivorous rodents have become increasingly popular as pocket pets in homes in many countries around the world including Japan, Germany, Italy, Belgium and the United States (Kerekes, 2007) over the past couple of decades. Veterinarians who treat these nontraditional pets have a difficult time determining the level of individual health due to lack of baseline normal data.

The term “captive” is used in this paper to describe prairie dogs that are kept as pets, in zoos, and nature centers that feed and protect/care for their prairie dogs. The term “pet” is used in this paper to describe those captive prairie dogs that have been properly maintained and cared for in a home environment with a diet that mimics that of prairie dogs living in the wild. These captive prairie dogs are fed a tremendous variety of foods, and blood is usually only drawn when health problems occur. Whereas some owners/facilities are strict with following proper dietary menus and portions, others are lenient. Also, the term “wild” is used in this paper to describe prairie dogs that have been recently caught in the wild and then used in research.

One goal of this study was to establish baselines for healthy, pet prairie dog blood cell counts and blood chemistry. Results from this study should represent a

realistic range of values for veterinarians to reference. An additional goal of this study was a statistical comparisons of this pet prairie dog data set with a data set established using wild prairie dogs collected for Broughton (1992) for use in gallbladder research.

In June, 2003, the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) issued a joint order banning the transport, sale, distribution, and/or release of prairie dogs into the environment within the United States without a special-issued federal permit (Federal Register, 2003). This ban was issued in response as a preventative measure to stop the spread of monkey pox, which occurred for the first time within the United States that same year (Federal Register, 2003). Although monkey pox was only documented in pet prairie dogs (as opposed to wild populations), these animals were no longer legally marketable as pets in the U.S. Prairie dogs that were already owned in captivity and not exposed to monkey pox were permitted to remain in their captive homes; however, the ban did apply to all other aspects in regards to the remainder of their captive lives and that of any future offspring.

Unfortunately, this study was not completed when the ban was issued, and the project fell short of the 45 count goal for the pet prairie dog study group due to lack of new pet participants as well as decreasing numbers of older pet participants. However, the data gathered thus far from this study (N = 20) will still aid veterinarians in treatment of any pet prairie dogs such as current pets (which can live up to 12 years; Kerekes, 2007), zoos, and nature centers, as well as for future pets if the ban is ever lifted.

Pet prairie dogs in this study were only included if they were deemed healthy by co-investigator and exotic pet veterinarian, Gregory Moore, and if their diet was consistent with standard veterinarian guidelines (Johnson-Delany, 1996; Kerekes, 2007)

which were designed to replicate the diet of prairie dogs living in the wild. These guidelines stress that the herbivorous prairie dog diet consist mainly of hay (such as Timothy hay, *Phleum pratense*), supplements of formulated, prairie dog food such as Oxbow™ Prairie Delight (Oxbow Hay Company, Murdock, NE), small portions of certain fresh/dried fruits and vegetables such as carrots or sweet potatoes, and occasional treats such as vegetarian dog food (Johnson-Delany, 1996; Kerekes, 2007). These guidelines were established based on observations of foraging individuals, stomach contents, and fecal composition collected from prairie dogs (Stockard, 1930; Kelso, 1939; Bonham and Lerwick, 1976; Fagerstone, 1982; Shalaway and Slobodchikoff, 1988; Uresk et al., 1988; Hoogland, 1995).

Wild prairie dogs used for research, e.g., gallbladder studies, are commonly caught in the wild, are of unknown age, and are kept for a two-week adjustment period while being fed rat/rodent chow (Broughton, 1992; Broughton, et al., 1996; Miki, et al., 1993) prior to blood collection.

Table II-1.1 lists percentages of crude protein, crude fat, and crude fiber for the representative main components of diets for both studies of pet and wild prairie dogs. Rat/rodent chow has a much higher percentage of fat and protein than hay or Oxbow™ Prairie Delight (Oxbow Hay Company, Murdock, NE) as well as a much lower percentage of fiber. A comparison of means between this pet prairie dog study and Broughton's (1992) study has been included with the hypothesis that a significant differences exists between pet and wild prairie dog blood hematology and blood chemistry.

Another hematological and blood chemistry study was done by Tell (1995) using 30 black-tailed prairie dogs from the National Zoological Park in Washington, D.C. Published results contained an average, a range and sample size for each test. A comparison between this current study and the Tell's study was not performed due to lack of statistical information available, e.g., no standard deviations.

TABLE II-1.1. Percentage of crude protein, crude fat, and crude fiber found in rodent chow, Oxbow™ Prairie Delight, and timothy hay.

Food type	Minimum Protein	Minimum Fat	Maximum Fiber
Rodent chow*	23.0	4.5	6.0
Prairie Delight†	10.0	1.1	32.0
Timothy hay†	7.0	1.5	30.0

*LabDiet® (labdiet.com)

†Oxbow™ Hay Company (Murdock, NE)

CHAPTER 2

MATERIALS AND METHODS

Drawing Blood

Pet prairie dogs included in this study were brought into the veterinarian's office for routine checkups, spaying/neutering, and other minor procedures that required general anesthesia of the prairie dog. Owners of these prairie dogs were asked to allow their pets to participate in the study, informed of the blood drawing procedure, and signed a waiver of release.

Most pet prairie dogs are stressed by strangers, and therefore, difficult to handle. To reduce stress and ease handling of the animal, general anesthesia was induced and maintained with isoflurane using a dog face mask. Once sufficient relaxation occurred, two to three milliliters (ml or cc) was drawn from either the jugular or anterior vena cava using a syringe. The collected blood was divided into two separate Microtainer® tubes (©Becton Dickinson and Company, Franklin, Lakes, NJ). One tube contained ethylenediaminetetraacetic acid (EDTA) for use in complete blood cell counts and differentials, and the other tube was without an additive for the remaining blood chemistry analyses. Tubes were sent to either Antech Diagnostics© (Irving, Texas) or Idexx Laboratories©, Inc. (Westbrook, ME) for analyses. A total of 44 blood variables were measured in 20 prairie dogs.

Statistical Analyses

Simple descriptive statistics such as the mean, standard deviation, and range were determined using SAS® software 9.1 .3 (SAS Institute, Cary, NC). Microsoft Excel®

software (Microsoft Corporation, Redmond, WA) was used to conduct independent *t* tests to compare differences on means between wild and pet prairie dog blood data. Significance was determined at $\alpha = 0.05$.

CHAPTER 3

RESULTS AND DISCUSSION

Blood was collected from 20 pet black-tailed prairie dogs with ages ranging from 1 to 4 years old with a mean ± SD age of 1.43 ± 0.99 (Table II-3.1). Weight ranged from 619 to 1370 g with a mean ± SD of 969 ± 195 (Table II-3.1). A comparison between genders showed no significant differences among variables. Baseline results for pet prairie dog blood chemistries are given in Table II-3.2 and baseline hematology values are given in Table II-3.3. Variables included in the pet prairie dog study but not the wild prairie dog study consist of globulin, albumin/globulin ratio, amylase, lipase, creatine phosphokinase, calculated osmolality, magnesium, neutrophil count, lymphocyte count, monocyte count, eosinophil count, and basophil count.

TABLE II-3.1. Weight and age of 20 pet, black-tailed prairie dogs (*Cynomys ludovicianus*).

Variable	Mean ± SD	Range
Weight (g)	969 ± 195	619 - 1370
Age (years)	1.43 ± 0.99	1.00 - 4.00

TABLE II-3.2. Blood chemistry values for pet, black-tailed prairie dogs (*Cynomys ludovicianus*).

Variable (units)	*N*	Mean ± SD	Range
Aspartate transaminase (IU/L)	20	37.5 ± 62.2	5.00 - 242
Alanine transaminase (IU/L)	20	43.9 ± 55.53	8.00 - 222
Total bilirubin (mg/dl)	20	0.16 ± 0.08	0.10 - 0.30
Alkaline phosphatase (IU/L)	20	125.0 ± 76.14	37.0 - 335
γ-Glutamyl transpeptidase (IU/L)	18	1.17 ± 0.51	0.00 - 2.00
Total protein (g/dl)	20	6.43 ± 0.54	5.40 - 7.50
Albumin (g/dl)	20	3.07 ± 0.31	2.40 - 3.60
Globulin (g/dl)	20	3.37 ± 0.56	2.50 - 4.60
Albumin / Globulin ratio	20	0.90 ± 0.28	0.00 - 1.30
Cholesterol (mg/dl)	20	130 ± 33.0	81.0 - 210
Blood urea nitrogen (mg/dl)	20	24.5 ± 7.10	12.0 - 37.0
Creatinine (mg/dl)	20	0.70 ± 0.14	0.50 - 1.00
BUN/Creatinine ratio	20	36.4 ± 13.4	17.0 - 62.0
Phosphorus (mg/dl)	20	6.32 ± 1.22	3.80 - 8.20
Calcium (mg/dl)	20	8.82 ± 0.43	8.20 - 9.90
Glucose (mg/dl)	20	152.3 ± 39.8	100 - 236
Amylase (IU/L)	17	133.5 ± 44.0	75.0 - 214
Lipase (IU/L)	17	84.6 ± 34.0	40.0 - 160
Sodium (mEq/L)	20	144 ± 2.80	140 - 150
Potassium (mEq/L)	20	4.97 ± 0.75	3.70 - 6.70
Sodium/Potassium ratio	20	29.7 ± 4.58	22.0 - 39.0
Chloride (mEq/L)	20	103 ± 3.30	97.0 - 111
Creatine phosphokinase (IU/L)	19	5150 ± 9550	284 - 31500
Triglyceride (mg/dL)	17	90.2 ± 65.7	28.0 - 225
Osmolalility, calculated (mOSm/kg)	17	304 ± 5.97	293 - 315
Magnesium (mEq/L)	17	2.24 ± 0.20	1.90 - 2.70

IU/L = International Units/Liter
mEq/L = milliequivalants/Liter
mOSm/kg = milliosmoles/kilogram water

TABLE II-3.3. Hematology values for pet, black-tailed prairie dog (*Cynomys ludovicianus*).

Variable (units)	*N*	Mean ± SD	Range
White blood cells (10^3/µl)	20	4.31 ± 1.93	1.40 - 9.50
Red blood cells (10^3/µl)	19	6.81 ± 0.61	5.90 - 8.10
Hemoglobin conc. (g/dl)	19	12.6 ± 1.11	10.4 - 15.3
Hematocrit (%)	20	38.7 ± 3.05	33.4 - 45.1
Mean cell volume (fl)	19	56.8 ± 3.80	48.0 - 64.0
Mean cellular hemoglobin (pg)	19	18.6 ± 1.33	16.0 - 21.7
Mean cellular hemoglobin conc. (%)	19	32.7 ± 1.43	30.0 - 35.5
Neutrophils (/µl)	20	2750 ± 1580	1040 - 7410
Neutrophil percentage (%)	20	62.6 ± 12.7	32.0 - 78.0
Lymphocytes (/µl)	20	1240 ± 728	252 - 2960
Lymphocyte percentage (%)	20	28.6 ± 12.5	14.0 - 58.0
Monocytes (/µl)	20	229 ± 161	29.0 - 585
Monocyte percentage (%)	20	6.45 ± 4.75	1.00 - 14.0
Eosinophils (/µl)	20	73.5 ± 140	0.00 - 616
Eosinophil percentagae (%)	20	1.95 ± 3.22	0.00 - 14.0
Basophils (/µl)	20	13.5 ± 23.1	0.00 - 88.0
Basophil percentage (%)	20	0.40 ± 0.60	0.00 - 2.00
Platelet count (10^3/µl)	18	463 ± 235	245 - 1190

In comparing the means between the wild and pet prairie dog blood results, significant differences ($\alpha < 0.05$) were shown for 62% (18 of the 29) variables (Table II-3.4) including aspartate transaminase, alanine transaminase, total bilirubin, albumin, cholesterol, calcium, triglyceride, neutrophils, and monocytes. Nine of those 18 variables showed highly significant differences ($\alpha < 0.001$). These variables include alkaline phosphatase, γ-glutamyl transpeptidase, total protein, blood urea nitrogen (BUN), creatinine, glucose, chloride, white blood cell count, and lymphocytes.

White blood cell count may be higher in wild prairie dogs due to increased levels of stress or parasites from the wild. Whereas the pet prairie dogs in this study were sensitized to daily human interaction, the wild prairie dogs were not. Also, the pet prairie dogs did not posses ectoparasites or any obvious endoparasites.

The diet fed to Broughton's wild prairie dogs (1992) consisted of rat chow, which contains higher levels of crude protein and crude fat as well as lower levels of crude fiber as compared to that of the 100% herbivorous diet fed to pet prairie dogs. This increase in intake levels of protein and fat may explain the significant differences in associated blood chemistry values such as aspartate transaminase, alkaline phosphatase, BUN, γ-glutamyl transpeptidase, creatinine, and total bilirubin.

TABLE II-3.4. Blood chemistry variables (mean ± SD) for pet (this study) and wild (Broughton, 1992) black-tailed prairie dogs. Probabilities are from independent t tests comparing means between pet and wild animals.

	Pet	Wild	
Variable (units)	*N* = 18 to 20	*N* = 45	*P*-value
Aspartate transaminase (IU/L)	37.5 ± 62.2	74.9 ± 9.3	< 0.002
Alanine transaminase (IU/L)	43.9 ± 55.5	25.0 ± 16.2	< 0.05
Total Bilirubin (mg/dl)	0.16 ± 0.08	0.34 ± 0.36	< 0.05
Alkaline phosphatatse (IU/L)	125 ± 76.1	73.7 ± 22.1	< 0.001
γ-Glutamyl transpeptidase (IU/L)	1.17 ± 0.51	10.1 ± 1.20	< 0.001
Total protein (g/dl)	6.43 ± 0.54	5.70 ± 0.80	< 0.001
Albumin (g/dl)	3.07 ± 0.31	2.50 ± 1.10	< 0.05
Cholesterol (mg/dl)	130 ± 33.0	104 ± 25.8	< 0.002
Blood urea nitrogen (mg/dl)	24.5 ± 7.10	32.0 ± 7.40	< 0.001
Creatinine (mg/dl)	0.70 ± 0.14	0.54 ± 0.17	< 0.001
Phosphorus (mg/dl)	6.32 ± 1.22	6.30 ± 0.90	> 0.5
Calcium (mg/dl)	8.82 ± 0.43	9.10 ± 0.40	< 0.02
Glucose (mg/dl)	152 ± 39.8	318 ± 89.6	< 0.001
Sodium (mEq/l)	144 ± 2.80	143 ± 4.20	> 0.20
Potassium (mEq/l)	4.97 ± 0.75	5.10 ± 1.00	> 0.5
Chloride (mEq/l)	103 ± 3.30	97.6 ± 4.40	< 0.001
Triglyceride (mg/dl)	90.2 ± 65.7	59.6 ± 37.6	< 0.05
White blood cell count (10^3/µl)	4.31 ± 1.93	6.30 ± 1.90	< 0.001
Red blood cell count (10^3/µl)	6.81 ± 0.61	6.60 ± 1.10	> 0.20
Hemoglobin conc (g/dl)	12.6 ± 1.11	12.0 ± 2.00	> 0.20
Hematocrit (%)	38.7 ± 3.05	36.5 ± 7.00	> 0.10
Mean cell volume (fl)	56.8 ± 3.80	55.1 ± 5.20	> 0.20
Mean cellular hemoglobin (pg)	18.6 ± 1.33	18.5 ± 1.90	> 0.5
Mean cellular hemoglobin conc (%)	32.7 ± 1.43	33.5 ± 2.20	> 0.10
Neutrophils (/µl)	2750 ± 1580	3900 ± 1600	< 0.01
Lymphocytes (/µl)	1240 ± 728	2200 ± 900	< 0.001
Monocytes (/µl)	229 ± 162	130 ± 110	< 0.01
Eosinophils (/µl)	73.5 ± 140	100 ± 130	> 0.20
Platelet count (10^3/µl)	463 ± 235	466 ± 157	> 0.5

IU/L = International Units/Liter
mEq/L = milliequivalants/Liter
mOSm/kg = milliosmoles/kilogram water

CHAPTER 4

SUMMARY

Comparisons of means of blood analyses between wild prairie dogs used for research and healthy pet prairie dogs revealed many significant and highly significant differences. These results demonstrate that pet prairie dog blood analyses should be referenced against hematology and blood chemistry baselines established using healthy prairie dog subject groups. Additionally, data gathered from wild prairie dog blood analyses should be referenced against a hematology and blood chemistry baseline established using wild prairie dog subjects on a similar diet.

With pet prairie dogs living up to 12 years in captivity (Kerekes,2007), future studies on pet prairie dog blood should include more subjects and contain more variation of prairie dog ages. With the addition of more subjects, significant differences in variables may also surface between genders.

APPENDIX A

RAW DATA FOR SECTION A: ESTABLISHING A BASELINE FOR MONITORING GENE DIVERSITY OF BLACK-TAILED PRAIRIE DOGS (*Cynomys ludovicianus*) IN TEXAS USING MICROSATELLITE LOCI

Allelic configuration for each prairie dog sampled from for Cochran County (COC). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
COC-01	18	14	15	10	7	10
	19	14	16	10	8	11
COC-02	14	15	15	10	6	11
	16	16	16	10	8	11
COC-03	16	13	13	10	8	11
	16	16	15	11	8	11
COC-04	17	16	13	7	8	11
	19	16	16	11	9	11
COC-05	14	16	16	9	8	9
	19	16	16	11	9	10
COC-06	14	16	13	10	8	7
	19	16	13	10	8	11
COC-07	14	15	13	9	8	7
	18	16	17	10	10	11
COC-08	17	15	13	10	6	11
	17	16	15	10	6	11
COC-09	14	16	13	10	6	7
	17	16	15	11	7	11
COC-10	14	14	14	10	6	11
	17	16	15	11	8	11
COC-11	16	14	15	10	6	10
	17	14	18	11	9	11
COC-12	14	14	13	11	7	10
	16	16	13	11	8	11
COC-13	16	14	15	11	8	10
	16	15	18	11	9	10
COC-14	18	15	15	9	6	11
	19	16	17	10	8	11
COC-15	14	15	17	9	6	11
	18	16	18	10	8	11
COC-16	16	13	13	10	8	7
	20	16	17	10	8	11

Allelic configuration for each prairie dog sampled from for Cottle County (COT). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
COT-01	15	16	16	10	10	10
	16	16	16	11	10	10
COT-02	16	16	14	9	8	10
	16	16	16	9	8	11
COT-03	16	13	16	9	10	10
	16	16	16	9	10	10
COT-04	15	13	16	9	8	10
	16	16	16	9	10	11
COT-05	16	13	16	9	10	10
	16	16	16	9	10	11
COT-06	16	11	14	9	8	10
	16	16	16	10	9	10
COT-07	16	16	16	9	8	10
	17	16	16	11	10	11
COT-08	16	16	13	9	8	10
	16	16	16	10	10	10
COT-09	16	16	16	9	8	10
	19	16	16	10	9	11
COT-10	16	16	16	9	8	10
	16	16	16	9	10	10
COT-11	20	13	14	11	8	10
	20	16	16	11	10	11
COT-12	16	16	13	9	8	10
	16	16	16	10	10	10
COT-13	16	16	14	9	10	10
	16	16	16	10	10	10
COT-14	16	13	16	9	10	10
	16	16	16	9	10	10
COT-15	16	16	16	9	8	10
	16	16	16	11	10	11
COT-16	16	11	14	9	8	10
	16	11	16	9	8	11
COT-17	16	16	14	10	8	10
	16	16	14	11	9	11
COT-18	15	14	14	9	8	10
	16	16	16	9	9	11
COT-19	16	13	16	9	8	11
	20	16	16	11	10	11
COT-20	15	14	14	9	9	10
	15	16	16	9	9	11
COT-21	15	16	14	9	8	10
	18	16	16	9	9	11
COT-22	15	16	16	9	10	11
	16	16	16	10	10	11

Allelic configuration for each prairie dog sampled from for Dalhart County (DAL). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
DAL-01	17	11	13	10	6	11
	18	16	13	10	9	11
DAL-02	15	16	13	7	8	7
	16	17	14	11	8	10
DAL-03	14	14	13	9	7	10
	17	14	13	10	7	11
DAL-04	17	16	17	8	6	10
	18	17	17	8	7	11
DAL-05	17	14	13	9	6	10
	17	17	16	9	8	11
DAL-06	17	14	17	8	5	11
	18	16	18	11	10	11
DAL-07	17	14	14	12	8	10
	17	16	14	12	8	11
DAL-08	16	14	13	8	7	9
	17	16	13	10	8	11
DAL-09	17	14	17	8	5	11
	18	16	18	8	6	11
DAL-10	17	14	14	8	6	11
	18	16	17	10	6	13
DAL-11	18	14	14	9	8	10
	20	16	17	9	9	10
DAL-12	14	11	13	8	6	10
	17	16	13	9	6	10
DAL-13	14	16	14	10	6	10
	16	16	17	10	8	10
DAL-14	14	14	14	10	8	9
	18	14	16	11	10	10
DAL-15	17	14	14	8	8	10
	18	14	17	11	9	10
DAL-16	17	16	14	10	8	10
	17	16	14	11	8	11
DAL-17	16	14	14	9	8	10
	18	16	19	11	8	13
DAL-18	14	14	13	8	6	10
	17	16	14	9	6	10
DAL-19	17	11	14	11	6	10
	20	14	18	11	8	10
DAL-20	14	14	14	10	8	9
	15	16	14	11	8	10
DAL-21	16	11	14	9	10	10
	18	16	14	11	10	10
DAL-22	17	16	17	8	6	10
	18	17	17	8	6	10
DAL-23	14	14	13	9	8	10
	17	14	14	12	10	11

Allelic configuration for each prairie dog sampled from for Hartly County (HAR). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A1	A115	D2	D12	D115
HAR-01	16	16	13	9	8	10
	17	17	15	10	9	12
HAR-02	17	14	17	10	6	10
	19	16	17	10	8	11
HAR-03	14	14	13	9	8	10
	14	16	14	11	8	11
HAR-04	17	14	13	9	8	12
	17	16	18	9	8	12
HAR-05	17	14	13	7	7	10
	17	16	16	7	8	11
HAR-06	16	16	13	10	6	10
	17	16	17	10	8	10
HAR-07	16	14	15	11	8	10
	17	17	16	11	9	11
HAR-08	17	16	13	8	7	9
	17	17	17	10	8	11
HAR-09	17	14	13	9	8	10
	17	14	18	9	8	11
HAR-10	14	16	18	9	6	11
	19	17	18	10	6	11
HAR-11	16	13	15	11	6	10
	17	17	16	11	8	11
HAR-12	14	16	13	8	8	10
	18	17	14	11	9	11
HAR-13	17	14	17	10	6	10
	17	16	18	10	8	10
HAR-14	17	14	13	10	6	10
	19	16	18	10	8	11
HAR-15	14	14	13	10	7	9
	16	17	13	10	10	10
HAR-16	17	13	13	9	8	10
	17	17	18	10	8	11
HAR-17	16	16	14	8	7	9
	18	17	14	8	10	10
HAR-18	17	14	14	11	7	9
	18	17	15	11	8	11
HAR-19	17	14	18	10	6	10
	17	16	18	10	9	12
HAR-20	16	16	13	10	7	11
	18	16	14	10	8	12

Allelic configuration for each prairie dog sampled from for Hemphill County A (HEMA). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
HEMA-01	19	14	15	10	6	10
	21	14	16	11	8	10
HEMA-02	19	14	14	11	8	10
	19	16	16	11	8	12
HEMA-03	14	15	13	10	6	11
	19	16	14	10	6	11
HEMA-04	16	16	13	9	6	7
	17	16	15	12	6	13
HEMA-05	17	14	14	10	6	11
	17	16	17	11	6	13
HEMA-06	19	16	14	10	8	10
	21	16	14	11	8	11
HEMA-07	19	14	16	10	6	10
	21	14	17	10	8	12
HEMA-08	14	11	13	10	8	10
	21	14	13	10	8	13
HEMA-09	19	16	15	8	8	11
	21	16	16	11	8	11
HEMA-10	18	14	16	10	6	11
	21	16	17	10	8	13
HEMA-11	17	14	13	12	6	10
	22	15	14	12	8	13
HEMA-12	19	16	13	11	6	11
	21	16	13	11	6	11
HEMA-13	21	14	13	8	8	8
	21	16	13	8	9	11
HEMA-14	18	10	16	10	6	10
	21	16	17	10	6	12
HEMA-15	19	14	13	10	8	10
	21	15	14	10	9	10
HEMA-16	17	14	13	8	6	10
	18	16	13	9	8	13
HEMA-17	21	14	13	8	6	11
	21	16	13	10	6	11
HEMA-18	17	16	13	10	6	11
	19	16	15	11	8	11
HEMA-19	14	14	13	8	8	11
	21	16	15	8	9	11
HEMA-20	17	10	14	10	6	9
	18	14	17	10	8	10
HEMA-21	17	16	13	10	6	11
	19	16	15	11	8	11

Allelic configuration for each prairie dog sampled from for Hemphill County B (HEMB). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
HEMB-01	14	15	14	10	6	10
	16	15	17	10	6	12
HEMB-02	14	16	15	8	6	10
	15	19	16	9	8	10
HEMB-03	14	16	13	8	6	9
	15	19	14	10	9	11
HEMB-04	14	16	13	8	6	11
	15	16	15	9	8	12
HEMB-05	14	15	15	9	6	11
	20	18	16	9	6	13
HEMB-06	15	16	13	8	6	10
	17	16	15	8	8	11
HEMB-07	16	16	14	10	6	11
	17	16	15	10	6	11
HEMB-08	14	15	14	9	6	10
	17	16	17	10	8	12
HEMB-09	14	15	14	9	6	11
	14	16	17	10	8	12
HEMB-10	14	14	15	9	6	8
	14	15	15	10	6	12
HEMB-11	14	15	14	10	6	10
	17	15	17	10	8	13
HEMB-12	14	16	15	9	6	11
	17	16	17	9	6	12
HEMB-14	14	16	13	8	6	9
	15	16	14	10	6	10

Allelic configuration for each prairie dog sampled from for Howard County (HOW). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
HOW-01	14	11	15	10	6	12
	18	16	16	11	8	12
HOW-02	14	11	14	9	8	11
	18	16	14	9	8	12
HOW-03	14	11	13	9	8	11
	14	16	14	10	9	11
HOW-04	17	16	14	10	8	12
	18	16	14	11	8	12
HOW-05	16	11	13	10	8	11
	18	15	14	10	8	12
HOW-06	14	16	13	10	6	11
	16	16	13	11	8	11
HOW-07	14	16	14	11	8	12
	15	16	16	12	8	12
HOW-08	18	11	14	9	6	11
	19	15	17	9	8	12
HOW-09	14	11	14	10	8	11
	14	11	14	10	8	11
HOW-10	16	15	14	10	8	12
	18	15	15	10	8	12
HOW-11	14	15	13	10	8	10
	14	16	16	10	9	11
HOW-12	18	11	14	9	7	11
	18	15	14	9	8	11
HOW-13	16	15	14	10	8	11
	18	15	15	10	8	11
HOW-14	14	16	13	10	6	12
	18	16	13	10	8	12
HOW-15	14	15	16	9	8	11
	18	15	16	11	8	12
HOW-16	18	16	14	9	8	11
	18	16	16	11	8	12
HOW-17	14	15	14	9	6	12
	17	16	16	10	10	12
HOW-18	18	15	14	9	8	7
	18	16	16	9	8	11
HOW-19	14	11	14	9	7	7
	18	16	14	9	7	11
HOW-20	14	15	14	9	7	11
	18	15	14	10	10	12
HOW-21	14	11	14	9	6	12
	17	15	16	10	10	12
HOW-22	14	15	14	10	6	10
	18	16	16	10	6	11

Allelic configuration for each prairie dog sampled from for Hudspeth County (HUD). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
HUD-01	19	16	15	9	6	11
	20	16	15	9	8	11
HUD-02	17	15	14	10	8	12
	20	16	15	10	10	13
HUD-03	19	16	15	9	8	11
	20	16	15	9	9	11
HUD-04	19	16	15	9	8	11
	19	16	15	9	8	11
HUD-05	19	16	13	9	8	11
	20	16	15	9	8	11
HUD-06	14	16	15	9	8	11
	20	16	15	9	9	12
HUD-07	19	16	13	9	6	11
	20	16	15	9	9	11
HUD-08	17	16	15	9	8	11
	19	16	15	10	8	11
HUD-09	17	16	15	9	8	11
	19	16	15	9	8	11
HUD-10	19	16	15	9	8	11
	19	16	15	9	9	11
HUD-11	14	16	14	9	7	11
	14	17	15	11	7	11
HUD-12	19	16	13	9	8	11
	19	16	15	9	9	11
HUD-13	19	16	15	9	8	11
	19	16	15	9	8	11
HUD-14	14	16	13	10	8	11
	19	16	15	12	8	11
HUD-15	19	16	13	9	8	11
	20	16	13	9	8	11
HUD-16	14	16	15	9	8	11
	14	16	17	10	8	11
HUD-17	19	16	13	9	8	11
	20	16	15	9	8	11

Allelic configuration for each prairie dog sampled from for Lubbock County A (LUBA). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
LUBA-01	18	11	13	9	6	11
	19	15	13	10	10	12
LUBA-02	19	11	13	9	8	7
	20	16	15	10	10	7
LUBA-03	17	15	13	9	8	11
	19	16	15	10	10	12
LUBA-04	16	11	13	9	8	9
	16	16	15	10	10	11
LUBA-05	17	11	13	9	8	7
	19	16	13	10	8	12
LUBA-06	17	11	13	10	6	11
	19	15	15	11	10	11
LUBA-07	18	11	15	10	6	11
	18	15	16	10	10	11
LUBA-08	17	11	13	7	6	11
	17	15	16	11	6	12
LUBA-09	16	15	13	9	6	11
	19	16	15	11	10	11
LUBA-10	18	15	13	12	8	7
	19	16	13	12	10	12
LUBA-11	19	11	13	9	10	11
	19	16	13	10	10	12
LUBA-12	18	16	13	9	8	11
	20	17	14	10	9	12
LUBA-13	18	15	13	9	6	11
	18	15	13	10	8	11
LUBA-14	17	11	13	10	8	11
	18	15	16	10	10	12
LUBA-15	16	15	13	7	8	12
	17	16	16	10	10	12
LUBA-16	18	15	13	10	10	9
	19	15	13	10	10	11
LUBA-17	16	15	15	9	8	11
	18	16	16	10	10	11
LUBA-18	16	15	13	10	8	7
	18	15	15	11	10	7
LUBA-19	16	15	13	10	6	11
	17	16	16	11	8	11
LUBA-20	16	11	13	10	8	11
	17	15	13	10	10	12
LUBA-21	17	15	13	8	6	11
	19	15	15	10	8	11

Allelic configuration for each prairie dog sampled from for Lubbock County B (LUBB). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
LUBB-01	13	14	13	10	8	7
	18	14	13	10	8	10
LUBB-02	16	14	16	10	8	7
	20	16	16	10	8	9
LUBB-03	14	14	13	10	8	7
	17	15	13	10	8	9
LUBB-04	14	14	13	10	8	9
	14	14	14	10	8	9
LUBB-05	13	15	13	9	8	9
	17	16	13	10	8	10
LUBB-06	16	14	13	10	8	11
	17	17	16	10	8	14
LUBB-07	13	16	13	9	8	9
	17	16	16	9	8	10
LUBB-08	17	15	13	10	9	7
	19	16	14	10	10	10
LUBB-09	13	14	13	10	8	9
	16	16	16	11	8	11
LUBB-10	13	15	13	10	8	9
	16	16	13	11	8	10
LUBB-11	16	14	ND	10	8	10
	20	14	ND	10	9	11
LUBB-12	14	14	13	10	8	7
	17	16	16	10	8	7
LUBB-13	14	14	13	9	6	10
	17	16	13	10	8	11
LUBB-14	17	15	13	9	7	10
	18	16	13	10	8	11
LUBB-15	13	14	13	10	8	9
	17	14	16	10	8	11
LUBB-16	14	15	13	10	8	10
	19	16	14	10	8	11
LUBB-17	14	14	13	9	8	7
	17	16	13	10	8	9
LUBB-18	14	14	13	10	8	10
	17	16	14	10	10	11
LUBB-19	16	14	13	9	8	10
	18	14	13	10	8	11
LUBB-20	13	14	13	9	6	7
	18	15	13	9	6	10
LUBB-21	13	14	13	10	8	9
	16	16	16	10	8	11
LUBB-22	13	16	13	9	6	10
	18	16	16	10	8	11
LUBB-23	14	14	13	10	8	7
	14	15	13	10	8	10

Allelic configuration for each prairie dog sampled from for Lubbock County C (LUBC). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
LUBC-01	14	14	15	9	8	11
	15	16	17	9	8	11
LUBC-02	15	14	13	9	8	11
	19	16	13	10	8	13
LUBC-03	17	14	13	10	8	11
	19	15	14	12	10	13
LUBC-04	16	14	14	10	8	11
	19	15	14	10	10	13
LUBC-05	14	15	15	10	6	11
	17	16	17	12	8	11
LUBC-06	15	14	13	9	8	11
	16	16	13	10	8	12
LUBC-07	15	14	13	9	6	11
	19	15	13	10	8	11
LUBC-08	17	14	13	10	6	11
	20	16	16	10	6	11
LUBC-09	15	14	13	10	6	11
	20	17	15	10	8	11
LUBC-10	17	14	14	10	8	10
	17	16	17	10	8	11
LUBC-11	17	15	13	10	6	10
	17	16	13	12	6	10
LUBC-12	16	14	13	7	8	10
	16	17	14	10	8	11
LUBC-13	17	14	14	12	8	10
	19	17	14	12	8	11
LUBC-14	16	15	13	7	6	10
	17	15	16	12	8	11
LUBC-15	14	15	13	10	6	10
	17	16	16	12	8	11
LUBC-16	15	11	14	9	8	11
	19	14	17	10	8	11
LUBC-17	17	14	14	10	8	10
	17	16	17	10	9	11
LUBC-18	17	14	13	10	6	9
	18	16	16	10	6	11
LUBC-19	14	16	15	10	7	10
	17	16	17	10	8	10
LUBC-20	17	15	13	10	6	10
	19	16	14	10	9	11
LUBC-21	14	14	14	10	8	11
	17	16	17	10	8	12
LUBC-22	14	14	16	10	6	11
	17	15	17	12	6	11
LUBC-23	17	14	14	7	8	11
	17	15	15	9	8	13
LUBC-24	16	13	13	10	9	12
	17	16	16	10	9	14
LUBC-25	16	16	14	10	7	10
	17	16	17	10	8	11

Allelic configuration for each prairie dog sampled from for Lubbock County D (LUBD). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
LUBD-01	15	11	14	11	8	11
	16	16	17	11	10	11
LUBD-02	13	15	13	10	8	11
	17	16	17	11	8	13
LUBD-03	13	11	13	9	8	11
	17	15	16	10	10	11
LUBD-04	13	16	13	10	8	11
	17	17	13	11	8	11
LUBD-05	15	15	13	8	6	11
	15	15	16	8	8	11
LUBD-06	15	13	16	8	6	11
	17	15	16	11	6	11
LUBD-07	16	16	13	10	8	11
	17	16	16	10	8	11
LUBD-08	15	15	13	10	8	11
	17	16	16	10	10	11
LUBD-09	15	15	13	10	6	11
	17	16	17	10	6	11
LUBD-10	15	11	13	10	8	11
	17	16	16	10	8	11
LUBD-11	15	11	13	11	8	11
	16	16	14	11	8	11
LUBD-12	14	11	13	10	6	11
	16	15	13	11	10	11
LUBD-13	13	15	14	8	8	10
	15	16	17	8	8	13
LUBD-14	13	15	13	10	8	11
	17	17	14	11	8	13
LUBD-15	17	15	13	10	6	7
	17	16	17	10	6	11
LUBD-16	16	11	13	9	6	7
	16	15	16	10	8	8
LUBD-17	14	11	13	11	6	8
	15	13	13	11	8	10
LUBD-18	17	11	13	10	8	11
	17	11	13	10	8	11
LUBD-19	15	15	13	10	8	11
	17	16	13	10	10	11

Allelic configuration for each prairie dog sampled from for Lubbock County E (LUBE). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
LUBE-01	17	11	13	10	6	10
	19	15	17	10	9	13
LUBE-02	14	15	16	10	6	11
	15	15	17	10	9	11
LUBE-03	17	16	14	11	6	11
	17	16	16	11	8	13
LUBE-04	16	15	13	7	6	11
	17	16	14	11	6	11
LUBE-05	17	11	16	11	8	11
	17	15	16	11	9	13
LUBE-06	17	11	16	9	8	11
	19	16	17	10	8	11
LUBE-07	16	11	13	10	6	11
	17	16	14	11	8	13
LUBE-08	14	15	13	10	6	11
	17	15	16	10	8	11
LUBE-09	17	15	13	11	8	11
	17	16	14	11	9	13
LUBE-10	14	11	13	10	9	12
	17	16	13	11	10	13
LUBE-11	17	11	16	9	6	11
	19	16	16	10	8	11
LUBE-12	17	11	13	10	8	10
	17	16	16	10	8	11
LUBE-13	16	11	16	10	6	11
	17	16	16	10	9	12
LUBE-14	17	15	13	8	8	11
	19	15	16	11	8	13
LUBE-15	14	11	13	10	6	11
	16	14	17	11	6	13
LUBE-16	16	14	13	10	6	11
	17	16	16	10	6	11
LUBE-17	16	11	13	10	8	11
	17	16	16	11	9	13
LUBE-18	16	16	14	9	6	11
	17	16	16	10	6	13
LUBE-19	17	16	13	10	6	11
	17	16	13	11	10	12
LUBE-20	14	11	14	10	6	11
	17	11	16	11	8	13
LUBE-21	17	15	13	10	6	11
	17	16	16	10	6	13
LUBE-22	14	11	13	8	6	11
	19	15	16	10	8	13

Allelic configuration for each prairie dog sampled from for Pecos County (PEC). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
PEC-01	19	19	13	10	8	7
	19	19	17	11	8	12
PEC-02	17	12	13	10	6	8
	17	15	17	10	9	11
PEC-03	17	12	16	10	7	11
	19	14	17	11	8	11
PEC-04	17	19	16	9	8	11
	17	19	17	10	9	12
PEC-05	17	16	14	10	8	11
	19	16	17	11	9	11
PEC-06	17	16	13	10	8	11
	19	16	17	11	8	11
PEC-07	17	14	13	10	6	11
	17	16	14	11	9	11
PEC-08	17	14	13	10	7	11
	19	16	17	10	8	11
PEC-09	17	15	16	10	8	8
	17	19	16	10	9	11
PEC-10	17	12	13	10	6	11
	17	12	16	10	9	12
PEC-11	17	16	13	11	8	11
	19	19	17	11	8	11
PEC-12	17	14	13	10	6	11
	19	16	17	11	9	11
PEC-13	17	12	16	10	9	8
	17	15	17	10	9	11
PEC-14	17	14	14	10	6	11
	17	15	17	11	8	11
PEC-15	17	16	13	10	7	7
	19	19	17	11	8	11
PEC-16	17	14	16	10	8	8
	19	19	16	10	9	11
PEC-17	17	15	14	10	6	11
	19	16	17	11	9	11
PEC-18	17	14	14	10	8	11
	19	15	17	11	9	11
PEC-19	17	12	14	11	7	11
	17	14	17	11	8	11
PEC-20	17	16	13	10	8	11
	17	19	17	11	8	11
PEC-21	17	12	13	10	8	11
	17	12	16	10	9	12
PEC-22	17	12	13	10	9	8
	19	15	15	10	9	12

Allelic configuration for each prairie dog sampled from for Schleicher County (SCH). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
SCH-01	15	15	14	9	6	7
	17	16	16	10	9	11
SCH-02	17	15	13	9	6	11
	17	15	14	9	9	12
SCH-03	16	15	13	9	7	11
	17	16	15	10	7	11
SCH-04	17	16	14	9	6	11
	17	16	16	10	9	11
SCH-05	16	16	13	10	6	11
	17	16	14	10	6	11
SCH-06	16	16	13	11	6	10
	17	16	14	11	9	11
SCH-07	15	15	13	9	6	7
	17	17	14	9	6	12
SCH-08	17	14	13	11	6	12
	19	17	13	12	9	12
SCH-09	15	14	13	9	7	11
	16	16	14	9	7	11
SCH-10	15	12	13	9	7	10
	17	16	13	9	7	11
SCH-11	17	14	13	8	7	11
	17	16	13	9	7	11
SCH-12	15	15	13	9	7	11
	16	17	15	9	9	11
SCH-13	17	14	13	10	7	11
	19	15	14	11	9	12
SCH-14	17	12	13	9	8	11
	17	17	15	9	9	12

Allelic configuration for each prairie dog sampled from for Tarrant County (TAR). Numbers represent the number of nucleotide repeats found for each locus.

	Locus					
	A1	A111	A115	D2	D12	D115
TAR-01	14	11	ND	9	9	7
	15	11	ND	9	9	7
TAR-02	15	11	16	9	10	7
	16	14	16	10	10	11
TAR-03	15	14	13	9	9	7
	16	14	16	10	9	11
TAR-04	14	14	16	9	9	7
	15	14	16	10	10	11
TAR-05	15	11	16	9	9	7
	15	14	16	9	10	7
TAR-06	14	11	16	10	9	7
	16	14	16	10	9	7
TAR-07	15	11	13	9	9	7
	16	14	16	10	9	7
TAR-08	14	11	16	10	8	7
	15	14	16	11	8	7
TAR-09	15	11	16	9	9	7
	16	14	16	10	9	7
TAR-10	16	11	15	10	9	7
	16	14	16	10	10	7
TAR-11	15	14	16	9	9	7
	16	14	16	10	10	11
TAR-12	15	11	13	9	9	7
	16	14	13	10	10	7
TAR-13	14	11	13	8	9	7
	16	14	13	10	9	7
TAR-14	14	11	13	10	9	7
	14	14	16	10	10	7
TAR-15	14	11	13	9	9	7
	15	14	16	10	10	7
TAR-16	14	14	13	10	9	7
	16	14	13	10	10	7
TAR-17	15	11	13	9	9	7
	16	14	16	10	10	7
TAR-18	14	14	13	10	9	7
	16	14	16	10	9	7
TAR-19	14	11	16	10	9	7
	15	14	16	11	9	7

APPENDIX B

RAW DATA FOR SECTION B: ESTABLISHING HEMATOLOGY AND BLOOD CHEMISTRY BASELINES FOR CAPTIVE BLACK-TAILED PRAIRIE DOGS (*Cynomys ludovicianus*) AND COMPARING THIS BASELINE WITH A PREVIOUSLY ESTABLISHED BASELINE FOR WILD BLACK-TAILED PRAIRIE DOGS

Blood chemistry values for pet, black-tailed prairie dogs (*Cynomys ludovicianus*).

	Pet prairie dog subject									
Variable (units)	1	2	3	4	5	6	7	8	9	10
Aspartate transaminase (IU/L)	8.0	16	18	28	9.0	85	175	242	45	6.0
Alanine transaminase (IU/L)	15	33	17	62	18	64	222	166	36	47
Total bilirubin (mg/dl)	0.1	0.1	0.2	0.3	0.1	0.2	0.1	0.1	0.1	0.1
Alkaline phosphatase (IU/L)	37	116	112	126	103	85	105	152	191	51
γ-Glutamyl transpeptidase (IU/L)	1.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0
Total protein (g/dl)	6.6	7.1	6.0	7.0	6.7	6.2	5.6	5.4	6.0	6.2
Albumin (g/dl)	2.4	2.9	3.0	3.0	3.6	2.8	2.9	2.7	2.9	2.8
Globulin (g/dl)	4.2	4.2	3.0	4.0	3.1	3.4	2.7	2.7	3.1	3.4
Albumin / Globulin ratio	0.6	0.7	1.0	0.8	1.2	0.8	1.1	1.0	0.9	0.8
Cholesterol (mg/dl)	132	118	172	152	84	135	107	127	127	81
Blood urea nitrogen (mg/dl)	19	25	15	28	27	34	25	20	27	31
Creatinine (mg/dl)	1.0	0.5	0.8	0.8	0.8	0.6	0.5	0.5	0.6	0.7
BUN/Creatinine ratio	19	50	19	35	34	34	50	40	45	44
Phosphorus (mg/dl)	6.5	3.8	6.0	6.1	7.4	5.9	7.7	8.2	6.4	6.8
Calcium (mg/dl)	9.0	8.8	6.0	6.1	7.4	5.9	7.7	8.2	6.4	6.8
Glucose (mg/dl)	141	102	146	103	171	100	166	107	130	202
Amylase (IU/L)	117	124	179	171	214	87	114	84	75	184
Lipase (IU/L)	77	82	40	141	160	96	84	56	77	113
Sodium (mEq/L)	142	141	143	147	147	142	142	140	145	141
Potassium (mEq/L)	4.0	4.6	5.2	4.7	4.9	4.7	4.9	5.1	4.8	5.5
Sodium/Potassium ratio	36	31	28	31	30	30	29	27	30	26
Chloride (mEq/L)	102	98	104	106	111	105	101	97	104	100
Creatine phosphokinase (IU/L)	284	447	874	1268	351	14710	27640	31510	12700	804
Triglyceride (mg/dL)	76	47	51	61	195	61	176	41	35	225
Osmolalility, calculated (mOSm/kg)	299	297	299	310	313	302	302	293	307	304
Magnesium (mEq/L)	2.4	2.0	2.2	1.9	2.2	2.3	2.7	2.3	2.3	2.0

IU/L = International Units/Liter
mEq/L = milliequivalants/Liter
mOSm/kg = milliosmoles/kilogram water

Blood chemistry values for pet, black-tailed prairie dogs (*Cynomys ludovicianus*).

	Pet prairie dog subject									
Variable (units)	11	13	14	15	16	17	18	19	20	22
Aspartate transaminase (IU/L)	11	13	15	16	5.0	9.0	8.0	10	14	16.0
Alanine transaminase (IU/L)	68	10	26	9.0	11	19	9.0	8.0	26	12
Total bilirubin (mg/dl)	0.1	0.1	0.2	0.1	0.2	0.2	0.3	0.1	0.3	0.1
Alkaline phosphatase (IU/L)	287	112	159	59	63	137	335	43	81	146
γ-Glutamyl transpeptidase (IU/L)	2.0	1.0	1.0	2.0	2.0	1.0	n/a	1.0	n/a	0.0
Total protein (g/dl)	7.5	6.7	6.0	6.5	6.4	6.7	5.8	6.5	6.5	7.2
Albumin (g/dl)	2.9	3.5	3.2	3.2	3.3	3.2	3.3	3.4	2.8	3.5
Globulin (g/dl)	4.6	3.2	2.8	3.3	3.1	3.5	2.5	3.1	3.7	3.7
Albumin / Globulin ratio	0.6	1.1	1.1	1.0	1.1	0.9	1.3	1.1	0.8	0.9
Cholesterol (mg/dl)	188	129	210	121	138	136	92	97	109	145
Blood urea nitrogen (mg/dl)	25	15	25	12	23	37	29	21	36	15
Creatinine (mg/dl)	0.6	0.8	0.6	0.7	0.9	0.6	0.7	0.7	0.7	0.8
BUN/Creatinine ratio	42	19	42	17	26	62	41	30	37	19
Phosphorus (mg/dl)	6.8	6.2	5.7	7.1	5.1	6.2	5.0	7.3	3.9	8.2
Calcium (mg/dl)	9.2	9.0	9.1	9.4	8.4	8.8	8.6	8.2	9.0	9.9
Glucose (mg/dl)	150	136	142	236	197	117	206	143	207	143
Amylase (IU/L)	134	78	166	112	160	182	n/a	88	n/a	n/a
Lipase (IU/L)	115	58	101	53	41	91	n/a	54	n/a	n/a
Sodium (mEq/L)	142	150	149	143	143	143	145	145	147	146
Potassium (mEq/L)	6.0	5.7	3.8	3.7	6.0	4.9	4.3	4.8	6.7	5.0
Sodium/Potassium ratio	24	26	39	39	24	29	34	30	22	29
Chloride (mEq/L)	100	100	105	105	104	105	106	103	106	101
Creatine phosphokinase (IU/L)	1419	1387	701	1104	371	533	294	450	n/a	n/a
Triglyceride (mg/dL)	206	90	53	28	77	71	n/a	40	n/a	n/a
Osmolalility, calculated (mOSm/kg)	301	313	315	303	305	306	n/a	305	n/a	n/a
Magnesium (mEq/L)	2.5	2.2	2.4	2.0	2.2	2.1	n/a	2.3	n/a	n/a

IU/L = International Units/Liter
mEq/L = milliequivalants/Liter
mOSm/kg = milliosmoles/kilogram water

Weight, age and hematology values for pet, black-tailed prairie dog (*Cynomys ludovicianus*).

	Pet prairie dog subject									
Variable (units)	1	2	3	4	5	6	7	8	9	10
Weight (g)	619	659	802	1063	1367	898	1158	709	845	1284
Age (years)	1	1	1	1	1	1	1	1	1	4
White blood cells (10^3/µl)	4.4	6.3	3.1	1.7	4.5	5.6	7.1	9.5	5.9	3.9
Red blood cells (10^3/µl)	6.0	6.3	7.6	6.9	7.2	6.8	6.4	6.5	8.1	5.9
Hemoglobin conc. (g/dl)	11.2	11.9	14.1	12.5	14.0	12.5	12.2	10.4	13.5	12.0
Hematocrit (%)	35	37	43	39	42	40	39	35	44	37
Mean cell volume (fl)	59	58	56	56	58	59	61	54	55	62
Mean cellular hemoglobin (pg)	18.9	18.8	18.5	18.1	19.4	18.5	19.1	16.1	16.7	20.3
Mean cellular hemoglobin conc. (%)	32	32	33	32	34	32	31	30	30	33
Neutrophils (/µl)	1408	3150	1829	1071	3195	1088	4899	7410	3953	2340
Neutrophil percentage (%)	32	50	59	63	71	73	69	78	67	60
Lymphocytes (/µl)	2112	2961	1116	340	720	1456	2130	1900	1829	975
Lymphocyte percentage (%)	48	47	36	20	16	26	30	20	31	25
Monocytes (/µl)	176	189	155	238	585	56	71	190	118	390
Monocyte percentage (%)	4	3	5	14	13	1	1	2	2	10
Eosinophils (/µl)	616	0	0	34	0	0	0	0	0	156
Eosinophil percentagae (%)	14	0	0	2	0	0	0	0	0	4
Basophils (/µl)	88	0	0	17	0	0	0	0	0	39
Basophil percentage (%)	2	0	0	1	0	0	0	0	0	1
Platelet count (10^3/µl)	362	325	372	388	424	645	741	721	443	383

Weight, age and hematology values for pet, black-tailed prairie dog (*Cynomys ludovicianus*).

	Pet prairie dog subject									
Variable (units)	11	13	14	15	16	17	18	19	20	22
Weight (g)	1103	952	1043	1003	1056	1020	984	1100	798	922
Age (years)	3	1	1	1	1.5	1	1	1	4	1
White blood cells (10^3/µl)	4.5	2.9	1.8	3.9	3.6	4.2	3.6	3.2	1.4	5.0
Red blood cells (10^3/µl)	n/a	7.9	6.6	5.9	6.9	7.0	7.0	6.9	6.5	7.1
Hemoglobin conc. (g/dl)	n/a	12.6	12.5	11.5	12.7	13.2	12.9	12.4	11.9	15.3
Hematocrit (%)	40	38	37	36	38	40	38	38	33	45
Mean cell volume (fl)	n/a	48	56	61	55	57	55	55	51	64
Mean cellular hemoglobin (pg)	n/a	16.0	18.9	19.5	18.5	18.9	18.3	18.0	18.3	21.7
Mean cellular hemoglobin conc. (%)	n/a	33	34	32	34	33	34	33	36	34
Neutrophils (/µl)	3240	1363	1044	2496	2232	2730	1332	2368	1050	3800
Neutrophil percentage (%)	72	47	58	64	62	65	37	74	75	76
Lymphocytes (/µl)	1215	1392	486	819	792	798	2088	448	252	950
Lymphocyte percentage (%)	27	48	27	21	22	19	58	14	18	19
Monocytes (/µl)	45	29	216	429	432	504	144	320	98	200
Monocyte percentage (%)	1	1	12	11	12	12	4	10	7	4
Eosinophils (/µl)	0	116	36	117	108	168	36	32	0	50
Eosinophil percentagae (%)	0	4	2	3	3	4	1	1	0	1
Basophils (/µl)	0	0	18	39	36	0	0	32	0	0
Basophil percentage (%)	0	0	1	1	1	0	0	1	0	0
Platelet count (10^3/µl)	n/a	292	526	245	304	279	1193	n/a	266	426

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